THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special

meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The accounts of the Marine Biological Laboratory for the year 1947 have been audited as heretofore by Messrs. Seamans, Stetson and Tuttle, certified public ac-

countants of Boston, and a copy of their audit is available for inspection at any time in the Laboratory office.

There were few changes in the Balance Sheet from the preceding year, and it is appended as Exhibit A. As of December 31, 1947, the total *book* value of all the Endowment Assets, including the Scholarship Funds, was $978,677.67, an increase of $1,478.37. The securities and cash comprising these assets had at the end of the year a *market* value of $989,814.16. Plant Assets (Land, Buildings and Equipment) amounted to $1,311,950.55, a reduction for the year of $1,013.55. Current Assets were increased $10,156.31 to a total of $231,008.19. Current Liabilities (Accounts Payable) were $11,621.59.

In recent years the Treasurer's Report has also included the "Exhibit B—Income and Expense" of the Auditors' Report. This year there is being substituted a summary of "Receipts and Expenditures" prepared by the Treasurer giving the actual cash transactions during the year. The Auditors' statement of "Income and Expense" is a necessary accounting of the books of the Laboratory as they are set up, but does not, in the Treasurer's judgment, give a simple picture of the moneys received and spent during the year. It includes items of a purely bookkeeping character such as depreciation, the value of Library serials received through exchange, and interdepartmental charges. It does not include expenditures for capital items including some apparatus, books and serials for the Library, etc., even though some of these are normal operating expenses, nor either pensions paid nor funds paid into the Retirement Fund. (All of these are accounted for elsewhere in the Auditors' Report, Schedule IV or Exhibit B.) It does include all gifts in "income" even though some of these gifts are designated for such special purposes as a new boat, and cannot be used for current expenses.

Statement I which follows is a summary of the actual financial transactions of the year except for donations for special purposes, special agency accounts, and real estate development accounts which are listed subsequently. Actual receipts in 1947 for current operations were $250,098.72. Current expenditures were $253,822.09 and are listed in detail by departments in "II. Current Expenses." An additional $32,806.56 was spent on repairs and special purchases entered on the books as "Plant Assets" and listed below in "III. Additions to Capital Assets from Current Funds." The total of the year's expenditures is therefore $286,628.65. After the deduction of $2,671.07 transferred from the Carnegie Book Fund and used for the purchase of some of the Library items, the resulting cash deficit for the year is $33,858.86. This was taken care of by using $10,000 of the Reserve Fund (reducing it to $6,218.88) and by reducing the cash balances in the checking accounts to a total of $7,949.16 at the end of the year.

It should be noted that at the end of the year there was a net increase of the excess of Accounts Receivable over Accounts Payable amounting to $5,776.01, and an increase in the value of Supply Department inventory of $4,848.29. If these two items are taken into account, the actual deficit for the year is reduced to $23,234.56. On the other hand, if the reserve for depreciation is deducted, as it properly is in the Auditors' Report, the deficit would be $25,806.10 greater or $49,040.66.

1. *Cash Statement for Year Ended December 31, 1947*

	Receipts	Expenditures: Current	Expenditures: Additions to Capital Assets	Expenditures: Total Expenditures
Membership Dues	$ 2,238.00			
Donations for Current Expenses [1]	944.95			
Income from Endowment	35,616.77			
Income other Securities	21,492.00			
Real Estate Rentals	6,360.00	$ 1,002.84		$ 1,002.84
Instruction	12,630.00	7,824.94		7,824.94
Research (incl. Apparatus and Chemical Depts.)	25,193.34	17,372.60	$ 2,127.22	19,499.82
Mess	32,029.42	33,965.04	1,880.93	35,845.87
Dormitories and Apt. House	16,600.10	13,570.83	11,013.25	24,584.08
Library [2]	3,000.00	11,649.19	9,153.70	20,801.89
Buildings and Grounds		46,184.88	5,882.06	52,066.94
Supply Department [3]	87,462.76	86,551.69	2,749.50	89,301.19
"Biological Bulletin"	6,044.42	9,812.79		9,812.79
Administration		24,336.70		24,336.70
Miscellaneous	486.96	1,551.59		1,551.59
	$250,098.72	$253,822.09	$32,806.56	$286,628.65

Total Expenditures	$286,628.65	
Total Receipts	250,098.72	
		36,529.93
Deduct Carnegie Book Fund Payment		2,671.07
Deficit for Year		$ 33,858.86

None of the totals in the above Statement include any interdepartment charges, nor any charges for depreciation or interest on investment.

[1] Donations were $775 given by the "Associates" of the Laboratory for apparatus, and $169.95 contributed for a Washing Machine.

[2] The Library income of $3,000 is the payment from the Oceanographic Institution towards Library expenses. The monetary value of serials received in exchange for the "Bulletin," estimated at $4,937.80, is not included in the above, nor is the $1,350 received from the Oceanographic Institution for the purchase of books for their account.

[3] The actual sales of the Supply Department were $96,191.85. The values of specimens and supplies furnished Research and Instruction Depts. were $7,132.44 and $4,672.05 respectively. If these values are taken into account and also the gain in inventory of $4,848.29, the increase in accounts receivable of $5,233.85, and a debit charge of $1,800 for administrative and maintenance expense, there would be a net profit of $20,997.70 on the operations of the Supply Department for 1947. This does not take into account the $2,749.50 spent for capital items, or the Auditors' charges of $1,461.67 for depreciation and $2,221.29 for interest on investment. If these had been included, the net profit for the Supply Department would have been $14,565.24.

II. Current Expenses for 1947 by Departments

Administration		
Salaries		$ 18,851.55
Central Hanover Bank Trustee Commissions		1,034.87
Falmouth Nat'l Bank Service Charges		142.45
Audit		1,045.83
Treasurer's Office		600.00
Advertising		333.34
Office Supplies		837.74
Sundries (Telephone, Postage, etc.)		1,682.82
		24,528.60
Deduct Cash Receipts		191.90
		24,336.70
Instruction		
Salaries and Travel Allowances		7,273.80
Sundries		551.14
		7,824.94
Research (Incl. Apparatus and Chemical Depts.)		
Salaries		11,378.87
Travel		200.00
Repairs		798.67
Supplies and Sundries		6,763.96
		19,141.50
Deduct Cash Receipts		1,768.90
		17,372.60
Library		
Salaries		10,881.25
Office Supplies		423.29
Sundries		343.65
		11,648.19
Buildings and Grounds		
Salaries and Wages		22,630.19
Fuel		2,825.30
Gas		1,871.81
Light and Power		2,749.00
Water		623.88
Insurance		1,666.23
Repairs		10,605.74
Sundries		4,190.39
		47,162.54
Deduct Cash Receipts		977.66
		46,184.88
Dormitories and Apt. House		
Salaries and Wages		4,800.50
Lighting, Gas and Water		2,134.87
Repairs to Blds. and Grounds		2,820.28
Outside Rentals		500.00
Laundry		1,390.59
Insurance		734.22
Sundries		1,190.37
		$ 13,570.83
Mess		
Salaries and Wages		$ 8,431.22
Cost of Food		21,326.59
Gas, Water, Light and Power		1,650.78
Repairs		231.03
Replacement of Dishes, etc.		391.15
Insurance		603.69
Laundry		311.80
Freight and Express		102.07
Sundries		916.71
		33,965.04
Supply Department		
Salaries and Wages		30,756.78
Purchase of Specimens		38,668.92
Chemicals		2,579.60
Containers		3,708.63
Boat Expenses		1,972.44
Truck Expenses		825.69
Freight and Express		3,313.63
Fuel		775.37
Light		96.00
Office Supplies		511.18
Telephone and Telegraph		317.10
Insurance		873.74
Advertising		344.54
Specimens and Supplies purchased for Research		1,058.40
Sundries		749.66
		86,551.69
"Biological Bulletin"		
Salaries and Wages		2,101.00
Printing, etc.		7,711.79
		9,812.79
Real Estate (Rented)		
Taxes and Insurance on Bar Neck Property (Garage) and Janitor's House		1,002.84
Other Expenses		
Workmen's Compens. Ins.		725.28
Truck Expense		222.26
Bay Shore and Great Cedar Swamp Expenses		203.72
Interest on Mortgage		250.00
Evening Lectures		128.96
Special Repairs, 1944 Hurricane Damage		21.37
		1,551.59
Total Expenses		$253,822.09

III. Additions to Capital Assets from Current Funds

A. Land

Improvements Bake House Lot.	$ 201.00
	201.00

B. Buildings

Waterproofing of Dormitory	4,000.00
Waterproofing Apt. House	2,780.00
Dormitories	3,349.32
Brick Laboratory	2,116.75
Wharf	450.00
Other Buildings	1,081.14
	$13,777.21

C. Equipment

Apparatus Department	$ 2,127.22
Dormitories	883.93
Mess	1,880.83
Brick Laboratory	1,273.60
Carpenter Shop	347.62
Old Main Building	411.95
Supply Department	2,749.50
	9,674.65

D. Library

Back Sets	2,587.72
Books	801.85
Serials	3,901.46
Reprints	10.76
Binding	1,851.91
	9,153.70

Total Additions	$32,806.56

IV. Gifts for Special Purposes

A. Boat Fund

Contributions Received		$9,335.00
Payments on New Boat		4,032.21
Balance Dec. 31, 1947		5,302.79

In addition securities were received in 1947 from Mrs. W. Murray Crane for the Boat Fund that were subsequently sold for $784.53.

B. Dr. Frank R. Lillie Memorial Fund

Initial Contribution from Dr. G. H. A. Clowes $1,000.62

V. Real Estate Accounts

A. Devil's Lane Property

Cash Received in 1947 from Sale of Lots		$5,198.00
Disbursements:		
Taxes	$ 286.66	
Road Construction	4,161.66	4,448.32

Cost of Devil's Lane Property to Dec. 31, 1947 was $54,296.13. Eighteen lots were sold in 1946 and 1947 for $14,250.00. $6,990.50 was paid in 1946 and 1947 on these purchases. The Devil's Lane Property as of Dec. 31, 1947, was carried on the books at $40,046.13, with the unpaid installments on the lots sold amounting to $7,259.50 carried as Accounts Receivable.

B. Gansett Property

No cash transactions in 1947 except payment of $87.69 taxes. Gansett Property including Accounts Receivable of $970, and deducting Reserve of $1,950 is now carried on the books at $1,162.36.

VI. Agency Accounts

A. Fellowship Fund

Cash Received from the Lalor Foundation		$ 5,000.00
Disbursements:		
For Fellowships	$2,512.50	
For Apparatus, Supplies and Laboratory Space	1,444.47	3,956.97
Balance, Dec. 31, 1947		1,043.03

B. Cancer Research Account

Cash Received from U. S. Public Health Service as grant-in-aid for "The Mechanism of Cell Division and Protoplasmic Growth" (under direction of Dr. Robert Chambers)	$25,000.00
Cash paid for Salaries, Laboratory Space, Apparatus and Supplies	6,278.81
Balance, Dec. 31, 1947	18,721.19

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1947
(From Auditors' Report)

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 961,036.65	
Securities and Cash in Minor Funds		17,641.02	$ 978.677.67
Plant Assets:			
Land	$ 110,626.38		
Buildings	1,337,188.88		
Equipment	202,358.59		
Library	363,325.72		
	$2,013,499.57		
Less Reserve for Depreciation	722,069.52	$1,291,430.05	
Reserve Fund, Cash		6,218.88	
Book Fund, Securities and Cash		14,301.62	1,311,950.55
Current Assets:			
Cash		18,648.18	
Mortgage Note Receivable		2,425.00	
Accounts Receivable		39,100.94	
Inventories:			
Supply Department	$ 43,932.10		
"Biological Bulletin"	16,775.67	60,707.77	
Investments:			
Devil's Lane Property	47,305.63		
Gansett Property	1,162.36		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Securities	21,464.00		
Retirement Fund	16,388.44	99,020.43	
Prepaid Insurance		5,575.99	
Items in Suspense (Debits)		5,529.88	231,008.19
			$2,521,636.41

Liabilities

Endowment Funds:			
Endowment Funds	$ 959,619.12		
Reserve for Amortization	1,417.53	$ 961,036.65	
Minor Funds		17,641.02	$ 978,677.67
Plant Funds:			
Mortgage Note Payable		$ 5,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	134,386.51	1,306,950.55	1,311,950.55
Current Liabilities and Surplus:			
Accounts Payable		$ 11,621.59	
Items in Suspense (Credits)		1,937.38	
Reserve for Repairs and Replacements		7,166.71	
Current Surplus		210,282.51	231,008.19
			$2,521,636.41

Respectfully submitted,

DONALD M. BRODIE,

Treasurer

V. REPORT OF THE LIBRARIAN

1947

The sum of $11,500 appropriated to the library in 1947 was expended as follows: books, $588.11; serials, $3,936.94; binding, $1,851.91; supplies, $444.99; express, $117.52; salaries, $9,475.25 ($3,000 of this sum was contributed by the Woods Hole Oceanographic Institution); back sets, $130.39; insurance, $45.00; sundries, $247.11; total, $16,837.22. The cash receipts of the library totalled: for microfilms, $222.76 (the cost was $531.47); sale of duplicates, $264.20. These receipts revert to the Laboratory; so also do the fees from library readers. There were 55 of these readers in the library during the year.

Of the Carnegie Corporation of New York Fund, $2,457.33 was expended for the completion of 17 back sets and for the partial completion of 12 back set; $213.74 was expended for 10 books.

The Woods Hole Oceanographic Institution budget was $800 plus $500 for additional purchases made during the year. The total spent was $1,322.28. The Woods Hole Oceanographic Institution also contributed $3,000 (see above under "salaries").

During 1947, the library received 1,201 current journals: 327 (13 new) by subscription to the Marine Biological Laboratory; 48 (3 new) to the Woods Hole Oceanographic Institution; exchanges with the "Biological Bulletin," 512 (12 new; 43 reinstated foreign) and 130 (21 new; 3 reinstated foreign) with the Woods Hole Oceanographic Institution publications; 173 (8 new) as gifts to the former and 11

(3 new) to the latter. The library acquired 178 books: 55 by purchase of the Marine Biological Laboratory; 18 by purchase of the Woods Hole Oceanographic Institution; 9 by gift of the authors; 43 by the publishers; and 53 by various donors, those of Dr. Oscar W. Richards (8); Dr. W. J. V. Osterhout (1); Dr. Bradley M. Davis (2) and Dr. Paul S. Galtsoff (2) among the most notable. There were 32 back sets of serials completed; 19 purchased by the Marine Biological Laboratory (17 with the "Carnegie Fund"); 5 by the Woods Hole Oceanographic Institution; 6 by exchange with the "Biological Bulletin"; 2 by exchange with the Woods Hole Oceanographic Institution publications. Partially completed sets numbered 56: purchased by the Marine Biological Laboratory, 29 (12 with the "Carnegie Fund"); purchased by the Woods Hole Oceanographic Institution, 11; by exchange with the "Biological Bulletin," 1; by exchange with the Woods Hole Oceanographic Institution publications, 6; by gift and exchange of duplicate material, 9.

The reprint additions to the library were 6,926; current of 1947, 257; current of 1946, 1,041; and of previous dates, 5,628. Acknowledgment is made to Drs. F. A. Hartman, Charles Packard, and F. K. Knowlton for valuable contributions to the reprint collection. Also, through Dr. E. G. Butler of Princeton University, the reprint collection of Dr. Ulric Dahlgren was presented to the library.

At the end of the year 1947 the library contained 56,594 bound volumes and 149,218 reprints.

In the fall 96 titles of the German journals delayed since 1940 were received in scattered volumes and numbers. The majority of these were for the years 1940–42.

The above report for 1947 is given in a format similar to preceding years. There follows a summary of the main events in the growth of the library from 1924 to 1947 inclusive. The figures of the varying budgets and acquisitions have been transformed for easy reading into graphs covering these twenty-four years. The data, as well as the running account, have been gathered for the most part from the yearly reports of the librarian.

The year 1924 was chosen for the starting point for several reasons. For one, a fair account of the library from its inception in 1888 to 1924 has already been published in the "Collecting Net" of 1929. But more important, the date marks a change in the administration of the library, in its expansion and in its budget. Previous to 1924 there is no mention of a library committee. The librarian (Dr. H. McE. Knower, followed by Dr. R. P. Bigelow) directed the work with the help of a paid assistant from 1914 to 1923. In 1924 a committee was appointed by the trustees with Dr. C. E. McClung as chairman, and Mrs. T. H. Montgomery, Jr. was made the full-time librarian. Dr. McClung remained as chairman through 1924–1931. Professor E. G. Conklin followed until 1941 and Dr. A. C. Redfield served in this office from 1942 to the end of 1947. In 1924, also, the present library was under construction; and in 1925 the collection was moved from room 217 in the Crane Building to its present position. At this time the library acquired a secretary, Miss Deborah Lawrence, whose invaluable assistance has continued throughout the development of the library into the present. The special significance of the period beginning in 1924 consists in the change that occurred in the library budget. Careful checks having been made to list the journals and books necessary for research in an expanding library, large sums of money were secured to purchase these, especially the back sets of needed journals. The accumulated "library fund"

of $8,000 was made available for 1924–25. In 1925 the General Education Board contributed $50,000 on the condition that it should be spent for back sets during the years 1926–30. In 1929 the General Education Board gave $200,000 for the general purposes of the library.

In addition to these gifts there have been special funds which came to the library later, not a part of the regular annual budget. The Woods Hole Oceanographic Institution, established in 1930, in that year contributed $5,000 for back sets and books, and for current journals on oceanography. Subsequently its annual budget for this purpose was $1,000 to $600, increased in 1941 to $800. In addition it has contributed to our library salary budget. The appropriation for this purpose was $1,100 in 1944, $1,700 in 1946 and $3,000 in 1947. The "Carnegie Fund" of $25,000 became available in 1941. Since the gift was not conditioned by a time limit for its expenditure and since the market for rare sets has been limited during and after the war years there remains a balance of this fund amounting to about $10,000.

The spacious library, begun in 1924, with five floors of stacks sufficient to hold 100,000 volumes, large reading and cataloguing rooms and librarian's office was thought to be adequate for many years. As early as 1936, however, when the library had acquired half this number of volumes and about 100,000 reprints the librarian reported "it is necessary to explain also how 50,000 volumes (which will be the total in four years if growth occurs at the present rate of 2,000 annually, and and 108,000 reprints if each years adds 3,500) will completely fill space that in 1925 was estimated to be adequate for 100,000 volumes, or 20,000 on each of five floors. The reprint floor at once reduces the available space for volumes to a capacity of 80,000. Besides this the many serial sets and books of quarto size, and over, reduce the space, and half of the bound serials recorded in our count are in reality two volumes bound together, so that the library will at the end of the year 1940 actually be housing more nearly 75,000 volumes, counted as volumes and not by the accession number, and 108,000 reprints." This prediction was amply fulfilled. By 1940 all available space in the stacks and wall shelves was occupied. An appeal to the Rockefeller Foundation for funds to build an addition met with a generous response. This gift of $110,400 was used, during the fall and winter of 1940–41, to erect a new wing which more than doubled the capacity of the library. Special provision was made for readers who can now enjoy private, well-lighted tables.

Through the period from 1924 until 1941 Dr. Frank R. Lillie was President of the Corporation and Chairman of the Executive Committee of the Laboratory. Any analysis of the steps taken in the development of the library throughout these years points directly to his wisdom, forethought and executive ability. He conceived its broad plan and under his guidance the library has grown from a small beginning to its present outstanding position.

The cost of building up and maintaining the library is shown in Figure 1. The total expenditures for each year since 1924 are given, and the amounts used for serials and books, and for back sets. Other expenses, such as salaries, binding, supplies, etc., are not shown. Reference has already been made to the great increase in overall expenditure from 1924 to 1932. The effects of the depression then began to be felt. The income of the Library Endowment and of the Laboratory as a whole dropped sharply. The library budgets have been successively re-

duced. This is especially noticeable during the war years when foreign journals, those from Germany in particular, could not be delivered and paid for. The extent of the drop in the number of journals received at that time is seen in Figure 2. Beginning in 1944 the number has risen rapidly until now the total is 1,201, as contrasted with 1,339 in 1936. Some of the back numbers published during the war are gradually coming in.

The yearly additions of volumes of serials and reprints is shown in Figure 3. At present there are 56,594 such volumes and in addition 8,000 books. Attention is called to the reprint receipts from 1924 to 1932, an increase of nearly 7,000 each year. During these years the large collections from Dr. Whitman's library and

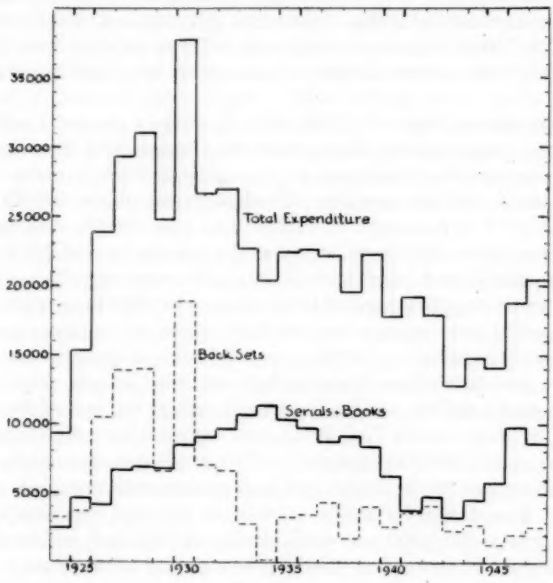

FIGURE 1

those of Sidney I. Smith and Maynard M. Metcalf were catalogued. At this time also about 5,000 reprints previously listed under books were shifted from the count of volumes to that of reprints. In 1938, besides other generous gifts, a collection from Dr. F. R. Lillie was recorded, and in 1943–1947 Dr. Rudolf Höber's large contribution, those of Drs. E. B. Meigs, W. E. Garrey, A. C. Redfield, Ulric Dahlgren, and others, greatly increased the count.

Much of the work of the Library Committee and of the staff has already been indicated. But in a summary of the past twenty-four years it is appropriate to note other services that are not as obvious from a study of the tables. That the purchase of journals and books was preceded by a careful selection is self-evident. In the matter of choice the investigators who use the library have always been the arbiters. To aid them in this the librarian, under the direction of the Library

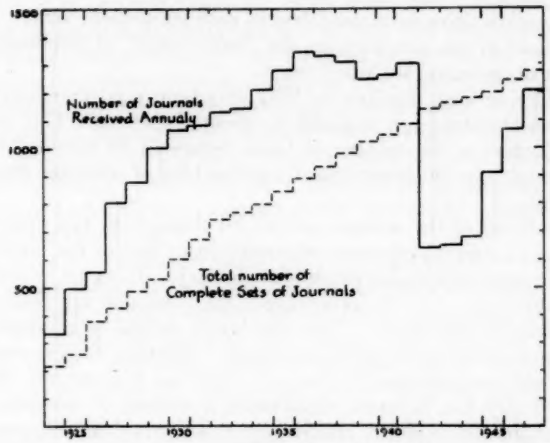

FIGURE 2

Committee has, each summer since 1927, compiled a list of desirable journals and books. The titles on these lists recommended by the investigators, as well as their own suggestions, were obtained either by purchase or exchange. Policies regarding exchange material were decided at the summer meeting of the Library Committee.

Another service rendered by the staff was the preparation of the complete list of journal titles, published as a supplement to the "Biological Bulletin." The full titles and holdings of each journal, with cross references of duplication in titles, were arranged in alphabetical order exactly as they appear on the shelves of the

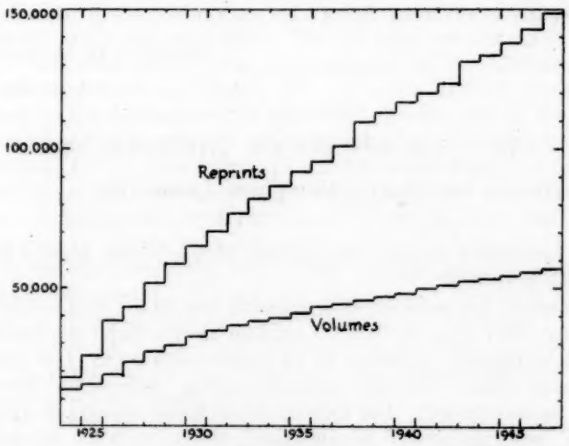

FIGURE 3

library. This made a volume of 80 pages in its initial publication in 1943. Additions, in similar form, have been published in each subsequent year.

For the benefit of the investigators the "sales room" of duplicate reprints and books has been in operation for many years.

The microfilm service, initiated in 1942, has been used extensively by investigators to secure literature not available to them elsewhere. This service has resulted in a reduction in the number of loans requested by outside libraries during the winter months. In this connection the printed list of journals, mentioned above, has been helpful.

The most difficult of the services carried on throughout this period was that of the catalogues. A journal catalogue separated from that of the books and reprints and complete in cross-references proved satisfactory. It seems to present no problem for its future use. On the other hand, the catalogue for books and reprints has always presented difficulties. That the books should be catalogued by subject as well as by author has never been questioned. Whether the enormous collections of reprints should be catalogued in the same way has not met the same unanimity of opinion. Finally the librarian established a system of assigning subjects by which the catalogue became less bulky. By this method a bibliography card was placed in front of each author's catalogued works. On this card a list of his subjects appears and on a subject card a cross-reference is given to the author's name. In this way many names appear as cross-references from the subject, thus eliminating the making of repetitive subject cards. After this system was installed throughout the catalogue, it was estimated that the card count was 472,500. If the new entries following 1947 can be continued in this manner the catalogue should form a useful guide to those unacquainted with the literature of the reprint collection.

This account of the library is not complete without a very special acknowledgment of the constant attention given to its development by the Directors (Dr. Merkel M. Jacobs and Dr. Charles Packard) and by the Library Committee members. All problems, large or small, in policy or in execution, that were laid before them, received attentive guidance and encouragement. Through their suggestions and moral support the work of the library has maintained its growth and stability.

PRISCILLA B. MONTGOMERY,
Librarian (retired)

VI. THE REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY

Gentlemen:

I submit herewith a report of the sixtieth session of the Marine Biological Laboratory for the year 1947.

The Laboratory has suffered an irreparable loss in the death of Dr. F. R. Lillie. For more than forty years he was its guiding spirit; under his leadership it grew from a small, struggling institution to its present eminence. His career began and ended in Woods Hole. Here he came as a young student, and here, full of years and honors, he was buried. For fifty-six consecutive seasons he returned, serving as instructor, investigator, and administrator, always with the interests of the Lab-

oratory at heart. On becoming Director in 1907 he said, "Our purpose is essentially ideal, and its pursuit demands our best efforts and our loyalty." When he resigned in 1926 the trustees wrote, "The Trustees appreciate the conspicuous ability, combined with unselfishness, with which you have guided the Laboratory. To you, in large measure, is due the steady broadening in scope and method that has been so conspicuous a feature of its work in recent years. As its Director you began with an institution already rich in achievement but still poverty stricken in respect to material things. You have left it unsurpassed in equipment and endowment, the center of activities that exert an always increasing influence on scientific progress throughout the world."

In his reply Dr. Lillie characteristically gave all credit to others—to the Trustees, to Mr. Crane, to the technical staff, and added, "Patience and faith were the only other necessary factors. I have no fear for the future as long as the Woods Hole spirit survives." Genuine cooperation and mutual helpfulness, he said, constitute the basis of this spirit. But elsewhere he added this note of warning, "We must not allow ourselves to forget that the principles for which we stand are never entirely won."

Those of us who worked with him will not forget his quiet and unhurried approach to current problems, his ability to foresee future needs, his untiring and successful efforts to meet them; and his lifelong devotion to the welfare of this Laboratory. It is fitting that a tablet to his memory should be placed in the entrance of the building. But more than that, we should build an F. R. Lillie Laboratory which will remind future generations of biologists of our debt to him.

1. Changes in Personnel

Mrs. Montgomery, our Librarian since 1924, retired at the end of 1947. For twenty-three years she carried on the business of the Library, beginning at the time when it was a small and insignificant collection, and continuing throughout the entire period of its expansion. In addition to her regular Annual Report she has presented a brief history of the Library during her incumbency. Of her own important and successful work she says little. But all who use the Library are fully aware that she, more than anyone else, is responsible for its completeness, its fine arrangement, and its perfect condition. We owe her a debt of gratitude for her outstanding work in the development of this world famous part of the Laboratory.

Miss Deborah Lawrence, who has worked with Mrs. Montgomery since 1925, has been appointed Acting Librarian. Under her supervision the Library continues to be carried on in a most satisfactory way.

Mr. Larkin, Superintendent of Buildings and Grounds, also retired this year. Through a long term of service he has been devoted to the interests of the Laboratory, on hand in emergencies at any time of day or night, always capably carrying on the work of his department. He has been retained as Consultant because of his intimate knowledge of the complicated installations in our buildings.

Mr. Robert Kahler, for many years Mr. Larkin's assistant, is now in charge of the plant, and has demonstrated his competence and resourcefulness in meeting the usual and unusual problems connected with the work.

Mr. Homer P. Smith joined the staff in July 1947 as Assistant Business Manager. The need of a second man in the office has been felt for some time for the

business of the Laboratory has increased greatly in recent years, and one man could not be expected to carry the entire burden. Under Mr. MacNaught's tutelage he has become familiar with the various phases of work, has adapted himself to the situation, and has proved to be a valuable addition.

2. New Boats

The Laboratory is now well provided with boats for all its various needs. New craft were urgently needed to replace the *Sagitta*, considered unsafe after 40 years of service, to collect the large amount of material required by schools and colleges, and to carry the classes on their field trips. It was, therefore, decided to make an appeal for funds to all members of the Corporation, to all workers at the Laboratory, and to friends living in the vicinity of Woods Hole. The Boat Fund Committee, under the direction of Dr. Heilbrunn, was highly successful in obtaining contributions, the total amount being $10,035. Among the nearly 300 donors were the Lilly Endowment $3,000, Mr. William Proctor $1,000, and Dr. G. H. A. Clowes $1,000.

Dr. Redfield, Chairman of the Committee appointed to secure a suitable boat, after visiting a number of boat yards, advised that the new craft should be designed by Mr. Crocker, a naval architect. After the Committee had approved the plans, construction was begun and carried forward without delay. The boat, 33 feet on the water line, has a cruising speed of 12 knots, and is equipped with dredging gear and a two-way radio. It will be in service in the summer of 1948.

In the spring of 1948 Mr. Crocker reported that a much larger boat, which he designed, could be bought for $12,000—about half the original cost. Mr. Walter Kahler inspected it thoroughly and urged that the Laboratory acquire it. The Executive Committee approved its purchase. The boat, large enough to accommodate the entire Zoology class, will be used during the summer of 1948.

3. Repairs and Improvements

In the course of the past two years we have made many repairs and improvements in several of our buildings. The much needed waterproofing, roof repairs, and outside painting of the Brick Building have been completed. The Apparatus Department is now situated in the well lighted and airy basement of the new wing of the Library. The rooms which were vacated will be now used as laboratories. The Chemical Department now has an air-conditioned room where special measurements can be made. The south wing of Old Main has been shored up so that the floors no longer vibrate as freely as in the past. Useful changes have been made in the Botany and Rockefeller Buildings. In the Stone Building a freight elevator is under construction. The top floor can be used for the storage of Supply Department materials. The Drew House has been put into good shape and painted; a new apartment was made out of the reception room of the Apartment House. Here also extensive repairs to the balcony supports were imperative. These and other much needed changes and repairs have cost nearly $38,000.

We must next make extensive alterations in the Supply Department Building, and put in good condition the wooden residences. Each year at least one of our buildings should be restored to first class shape. In this way they can be prevented from falling into serious disrepair.

4. The Housing Problem

Each year since the war ended we have experienced great difficulty in providing living quarters for those who want to work here. This is, to a considerable extent, due to the fact that, compared with former years, many more of the young investigators and students are married and require apartments, suites, or other special accommodations for their growing families. The Laboratory can provide for a relatively small proportion of these applicants; in the village there is a reluctance to rent rooms to families with children. As a result a number of good investigators are forced to withdraw their requests for research space.

When the housing situation became critical soon after the Brick Building was finished, Dr. Lillie urged the erection of a building to accommodate a large number of single investigators, and, in addition, ten bungalows for families. Today we can provide for the first group, but for the latter we need more apartments or small houses. To add to our present Apartment House or to erect a new one is too costly a venture at this time. But simple houses could be built on Devil's Lane property at a moderate cost. I believe that if the Laboratory built several, with a view to selling them to our members on easy terms, we could soon dispose of them, together with the lots on which they stand. Without doubt such a housing project would stimulate others to purchase Devil's Lane lots. Thus both the workers and the Laboratory would be benefited.

5. Lalor Fellowships

Lalor Fellowships, established by the Lalor Foundation, were granted to the following investigators:

Senior Fellow: Prof. Jean Brachet, University of Brussels, Visiting Professor at the University of Pennsylvania

Junior Fellows: Dr. I. M. Klotz—Northwestern University
Dr. Arnold Lazarow—Western Reserve University
Dr. Benjamin Libet—University of Chicago
Dr. Claude Villee—Harvard University

6. Winter Research

The Laboratory of Experimental Cell Research, under the direction of Dr. Robert Chambers, has been engaged since the Fall of 1947 in the study of the mechanism of cell division and growth, employing micromanipulation methods and tissue cultures.

7. Gifts

The Laboratory gratefully acknowledges the following gifts:
The Associates of the Marine Biological Laboratory, $775
Dr. G. H. A. Clowes—for a Lillie Memorial, $1,000
Mrs. W. Murray Crane, $700
Contributors to the Boat Fund, $10,035

8. Election of Trustees

At the Meeting of the Corporation on August 12, 1947, the following trustees were elected:

Class of 1951

W. C. Allee
C. L. Claff
G. H. A. Clowes
K. S. Cole
P. S. Galtsoff
L. V. Heilbrunn
J. H. Northrop
A. H. Sturtevant

9. There are appended as parts of this report:

1. Memorials to Prof. William B. Scott, Prof. Robert A. Harper, Mr. George M. Gray, Professor Herbert S. Jennings, Professor Samuel O. Mast, and Professor L. L. Woodruff
2. The Staff
3. Investigators and Students
4. Tabular View of Attendance
5. Subscribing and Cooperating Institutions
6. Evening Lectures
7. Shorter Scientific Papers (Seminars)
8. Members of the Corporation

Respectfully submitted,

CHARLES PACKARD,
Director

1. MEMORIALS

William Berryman Scott, 1858–1947

By E. G. Conklin

William Berryman Scott, the oldest member of the Board of Trustees of the Marine Biological Laboratory, was born on the birthday of Charles Darwin and Abraham Lincoln, February 12, but 49 years later than these great predecessors, viz. 1858. He died in his 90th year on March 29, 1947 in Princeton, New Jersey, his life-long home.

He and his colleague at Princeton, William Libbey, first visited Woods Hole in 1883 on invitation Spencer F. Baird, head of the U. S. Fish Commission, to confer with him on plans for the development of the Fisheries Station at Woods Hole, and as a result, Libbey contributed in the name of Princeton University, $1,000, toward the purchase of the land on which the Station was established.

In 1890, two years after the founding of the Marine Biological Laboratory, Prof. Scott became a member of the Corporation and in 1897 he was elected a Trustee and continued in that office and as Trustee Emeritus until his death. During these forty years as Trustee, he made it a point of honor to be present whenever possible at the annual meetings, and in 1897 and 1898, he gave lectures at the Laboratory on the methods and results of his paleontological researches.

Although he was for fifty years a member of the staff of the Department of Geology at Princeton, and for forty-six years head of that department, he was primarily a zoologist. At the close of his senior year in college, he and two other classmates, Osborn and Speir, organized a scientific expedition to Colorado and Wyoming. The collection of vertebrate fossils made that summer of 1877 was described in their first scientific publication and was instrumental in shaping the future careers of Scott and Osborn. After a year of graduate study at Princeton and a second expedition to the West in the summer of 1878, Scott spent two years in graduate study in Europe; first with Huxley in London, then with Balfour in Cambridge, and finally with Gegenbaur in Heidelberg. His work with these masters was in anatomy and embryology. Under the stimulus of Balfour, he, with Osborn, completed and published a study on "The Early Development of the Common Newt," the first such study on the embryology of a urodele. In Gegenbaur's laboratory he was assigned for study the valuable material which had been collected by Dr. Calberla, deceased, on the embryology of a cyclostome fish and the results of this study were published in Gegenbaur's Morphologisches Jahrbuch in 1880 as his thesis for the Ph.D. degree, with the title, "Beitrage zur Entwicklungsgeschichte der Petromyzonten."

Thus at the early age of twenty-two years, he had finished his work for the doctor's degree, published three important papers, been a leader in two exploring expeditions, and had met on terms of intimate friendship and cooperation, some of the foremost scientists and scholars of Europe and America.

On his return from Europe, he was appointed assistant in geology at Princeton and three years later was made full professor in that department, which position he continued to hold until he had completed fifty years of teaching at Princeton University. During that time, he made eight additional exploring expeditions to the West and published more than 150 paleontological reports. Perhaps his most monumental work was the "Reports of the Princeton Expedition to Patagonia" which was published in nine magnificent volumes, of which he was editor and co-author. His later work, undertaken after he was seventy-six years old, in association with his former student and colleague, Dr. Jepson, was a great monograph of 1,000 pages and 100 plates on the "Mammalian Fauna of the White River Oligocene"; while his latest work was a similar monograph on the "Mammalian Fauna of the Uinta Formation," upon the final pages of which he was at work until two days before his death.

In addition to these research publications, he was the author of a number of important books of a more general nature, among them, an "Introduction to Geology," which ran through three editions. "A History of Land Mammals of the Western Hemisphere," two editions; "Physiography, the Science of the Abode of Man"; "The Theory of Evolution"; and finally, a most interesting and important autobiography, "Some Memories of a Paleontologist."

He was abundantly honored both in Europe and America by universities and learned societies. The Universities of Pennsylvania, Harvard, Princeton and Oxford, gave him honorary degrees. He received ten medals and awards from learned societies here and abroad. He was elected a member of the American Philosophical Society when he was twenty-eight years old and at the time of his death, had been a member for more than sixty years. For seven years he was president of that soci-

ety of which his great, great, great-grandfather, Benjamin Franklin, had been founder and first president. He was also president of the Geological Society of America (1925) and of the Paleontological Society (1911), and a member of the National Academy of Sciences, the American and British Associations for Advancement of Science, the American Academy of Arts and Sciences, the Academy of Natural Sciences of Philadelphia, the New York Academy of Sciences, the Washington Academy of Sciences, the Geological, Zoological and Linnean Societies of London.

Professor Scott was a brilliant lecturer and he often enlivened scientific meetings with his humorous stories and his phenomenal memories of great men and events. Nevertheless, he was a scholar and thinker rather than a popular lecturer or writer. Fortunately, he has recorded in his autobiography many of his memories of some of the greatest men of his generation.

The Corporation and Trustees of the Marine Biological Laboratory record their sorrow in the loss of one of their oldest and most distinguished members and transmit to the members of his family this expression of their esteem and sympathy.

Dr. Robert A. Harper

By Edmund W. Sinnott

In Dr. Harper's death, plant science has lost a man who for years was one of its greatest leaders.

Dr. Harper was born on January 21, 1862 at Le Claire, Iowa. He received his B.A. at Oberlin in 1886 and then for two years taught Latin and Greek at Gates College. From 1889 to 1891 he was instructor in science at Lake Forest Academy. He took an M.A. at Oberlin 1891 and then served for a time as professor of botany and geology at Lake Forest College.

Dr. Harper's chief interests centered more and more in botany and he soon determined to make this his career. As so many young botanists did in the nineties, he went to Germany for graduate work. At Bonn he came under the influence of Strasburger and other notable teachers, taking his doctorate in 1896. Here began his life-long interest in cytology. Soon after his return to America, he went to the University of Wisconsin where he became professor of botany and head of the department. In 1911 he was called to Columbia University as Torrey Professor of Botany, serving until his retirement in 1930. Here he reorganized the department and greatly widened its scope and activities. He was keenly interested in the New York Botanical Garden, the Boyce Thompson Institute for Plant Research and the Torrey Botanical Club.

Dr. Harper was a student of plant cells since his days at Bonn, but he was much more than a mere cytologist. He liked to describe himself as a cellular physiologist and studied many aspects of the activities of all sorts of plant cells. He was for a long time especially concerned with a study of reproduction in the fungi, and his work on nuclear behavior in the ascomycetes is classic. Studies of development and morphogenesis particularly appealed to him and he was a stout supporter of the view that the phenomena of development are best approached through a knowledge of the behavior of cells. He was much interested in some of the simpler algae, notably Pediastrum and Hydrodictyon, in which he investigated the problems of cellular activities.

Unlike so many biologists of today, Dr. Harper had a wide knowledge not only of his own science, but of others and of wider fields of learning. He was a good field botanist, a successful farmer, a skilled experimenter, and a man of wide reading and erudition. He encouraged his students to train themselves broadly and not to be carried away by the fashions of the time. He was a stimulating teacher, and in a discussion, delighted to take the less popular side and to defend it vigorously. He was highly critical and a foe of slipshod works. His own papers were beautifully done and his drawings, in particular, were remarkably fine.

Dr. Harper had close contacts with Woods Hole for many years. He was a student here in 1891, along with Bradley Moore Davis, C. P. Sigerfoos and Katherine Foote. In 1893, he worked as an investigator at the Laboratory. Frequently during ensuing years he came to Woods Hole for part of the summer. He was elected to the Board of Trustees in 1911 and in 1932 became Trustee Emeritus. In recent time he has rarely come to the Laboratory but devoted all his time to his farm at Bedford, Virginia, where he spent his last years happily close to the soil and with plants he loved.

Dr. Harper's warm and friendly personality endeared him to his many students and to a host of friends all over the world. He was a great teacher, a friendly opponent in debate, and a constant stimulus and inspiration to all who knew him. He will be sorely missed.

George M. Gray

By W. C. Curtis

George Milton Gray was born at Bristol, Rhode Island, November 2, 1856. As a boy he was interested in natural history, particularly in birds and insects. When a young man he worked as a taxidermist, gave lectures on birds at a boys' camp and made collections in the vogue of his day. He was thus a naturalist from his youth up. Later, he was discovered by Dr. H. C. Bumpus and engaged as a technician in the Department of Zoology at Brown University. Mr. Gray first came to the Marine Biological Laboratory in the summer of 1891 as a laboratory assistant. He served in this capacity for six summers (1891–96), as collector for two summers (1897–98), and became Curator of the Supply Department in 1899.

Although this department was established in 1891, its effective operation began with Mr. Gray. At first the orders were filled only in the summer months. In the years 1896–97 and 1897–98, a stock was shipped to Williams College, and sales totaling about $125 per year were made during each of these winter periods. The trustees were elated when it was announced that the sales might exceed $500 for the summer of 1897 and again in 1898. In September 1899, when Mr. Gray became Curator and a year-round appointee of the Laboratory, the department began its continuous existence at Woods Hole. It was then located in the basement space between the two wings of the Old Main Building. In winter the supplies were moved to the invertebrate laboratory where a stove was set up to keep Mr. Gray and the specimens from freezing. The Stone Building, to which the department was transferred several years later, was luxury indeed when he was first established there. As a result of his untiring efforts during these early years, the department flourished. In the year 1912 the sales totaled $13,966.35. In 1925 the total was

$57,771.67. The creation of this necessary adjunct to the work of the Laboratory and important source of revenue was largely the work of George M. Gray.

Mr. Gray not only established the reputation of the department for reliability and for quality of material, he also established a record of service to classes and investigators. Beginning as a one-man organization, it employed an increasing number of assistants, and the role of those who "worked in the Supply Department" for a summer or two and later attained distinction in some field of biological science is an impressive one. Another notable contribution by Mr. Gray was his personal influence upon these youngsters.

In later years he was able to devote more time to aspects of the work in which he was particularly interested. After he withdrew from the Supply Department (1931), he became Curator of the Museum, which was named in his honor and was his special pride. He was fairly active even as Curator Emeritus since 1935. He died December 1, 1946 and is buried in the Woods Hole Cemetery.

Writing in a laboratory room, the windows of which look out upon the Hole and the islands where I worked as his assistant fifty years ago this summer, I cannot forbear my personal tribute. I have always thought him one of the most honest and kindly men I ever know. He was my close friend always from that summer long ago. About that time I was taken by a remark, made sadly by an elderly clergyman wise in the ways of men, that he had known some individuals to whom he thought the term "Christians" might be applicable although he had never seen any reason for applying it to church members as a group. I thought then and I have thought ever since that George Milton Gray was one of the few among my acquaintances to whom I would apply that term. We of the Marine Biological Laboratory never had a more devoted service nor greater loyalty than he gave us. He well exemplified the dictum of his faith: "He that would be great among you let him be the servant of you all."

Dr. Herbert Spencer Jennings

By O. C. Glaser

With the death of Herbert Spencer Jennings, at Santa Monica, California, on April 14, 1947, the Marine Biological Laboratory lost one of its most distinguished members—a trustee for 33 years and a trustee emeritus since 1938.

The son of a physician, Jennings was born in Tonica, Illinois, on April 8, 1868, and educated as he said "in most states of the Union." This migratory life continued with only short interludes until he was 38. Like his namesake, he was precocious and for years hard pressed by economic difficulties and the struggle for scientific opportunity. At 20 he prepared for college, so to speak, as an Assistant Professor of Botany and Horticulture at the Agricultural and Mechanical College of Texas. As an undergraduate at Ann Arbor, he joined the Biological Survey of the Great Lakes conducted by the Michigan State Board of Fisheries and laid the foundations for his first monograph on Rotifers, published in 1894—a year after receiving his B.S. from the University of Michigan. At Harvard he received the M.S. in 1895 and his Ph.D. in 1896. His thesis on cell-lineage of the rotifer Asplanchna herrickii, related the orientation of the spindles to the general problems of developmental mechanics whose solution he projected into the molecular realm.

Travelling fellowships enabled Jennings to spend the following year with Max

Verworn in Biedermann's Laboratory at Jena. Here began his preoccupation with the reactions of unicelluar organisms.

Returning in 1897, he became Professor of Botany and Bacteriology at the Agricultural College of Montana; accepted next instructorships, first at Dartmouth, then at Michigan where he became Assistant Professor of Zoology in 1900 and remained until called in 1903 to an Assistant Professor at the University of Pennsylvania.

These frequent translocations must have had serious disadvantages but his tremendous drive and unbounded enthusiasm found or created time for both teaching and research. By 1906 when he left Pennsylvania, he had to his credit at least 40 publications. The joint text with Reighard on the Anatomy of the Cat based on his own dissections and illustrated by Mrs. Jennings, belongs to the second Michigan period. Here too, as Director in 1901 of the Biological Survey of the Great Lakes under the U. S. B. F., he gathered material for three additional monographs on the Systematics of Rotifers and in connection with his teaching gave the first expression to a lively and recurrent interest in simulacra. Outstanding among the special contributions were his Psychology of the Protozoa in which Paramecium is shown to have "hardly taken the first step in the evolution of mind," and his analysis of the Biological Significance of Asymmetry. There were further studies on stimulation in Protozoa, followed by more from Pennsylvania. There were also publications on the behavior of the earthworm, the sea-anemone and the starfish—the latter a veritable storehouse of information on the activities of this animal—appearing in 1907. Many of his results during the Philadelphia period were either summarized in the great monograph on the behavior of lower organisms published by the Carnegie Institution or in the famous Behavior of the Lower Organisms appearing in the Columbia Biological Series in 1906; reprinted in 1915; and translated by Ernst Mangold in 1914 into German. The chief product of all his meticulous observations and simple experiments was a general outlook with variability, modifiability, unity and adaptiveness of behavior as the central themes.

With his transfer in 1906 to an Associate Professorship of Experimental Zoology at the Johns Hopkins University and his subsequent elevation to the Henry Walters Chair of Zoology and Directorship of the laboratory—both in succession to William Keith Brooks—Dr. Jennings entered upon his only long tour of duty at any one institution. He also changed his field of investigation. Although theoretical and controversial writings on behavior continued to appear, 1908 marks the beginning of a long series of researches on the life-cycles, heredity, variation, and evolution of Protozoa, notably Paramecium, Arcella, and Difflugia. In 1928, he reverted to his rotifers and, with Ruth Stocking Lynch, published two papers on Age, Mortality, Fertility, and Individual Diversities in Proales sordida.

During his genetic phase, Jennings substituted for full verbal description, long tables of measurements and enumerations. He became a biometrician. So highly did he perfect his mathematical techniques and insights that he was called upon to act as a statistician for the Food Administration during the first World War. Among his most important genetic results we must cite the analysis of conjugation in Paramecium whose significance he found in the diversities so created rather than in any rejuvenating effects; his pure lines in the same organism; and finally, the discovery of mating types—at long last the key to Protozoan Genetics. Through-

out this period, Jennings strengthened his position as the apostle of diversity. He also developed further his inborn sensitivities to the more general intellectual and social elimate. Contributions on special and general methodology such as genetic method and Radical Experimental Analysis appeared in a stream of critical evaluations of Vitalism, Mechanism, Determination and Freedom. In the social area, he wrote on Immigration, Defectives, "Undesirable Aliens," Racial Progress, the Family and Marriage. Apparently unaware of his powers, he invaded more distant territory. In his essay on the advantages of Growing Old, the euphorious state created by the presentation of a portrait of one's self and the pessimistic outlook of a young man trying to lead the life of a productive scholar on a $900 instructorship, are contrasted with humor, pathos and artistry sufficient to suggest pure literature. All told, the output of 32 years at Johns Hopkins amounts to about 120 papers, long or short, and seven books.

Modest, shy, nervous and frail, Jennings nevertheless accepted many outside lecture engagements. He spoke with great animation and charm to audiences invariably responsive to his sincere excitement and well ordered presentations. He gave more than one of our evening lectures. Few who heard his mathematical analysis of the data on genic linear array will forget his enraptured delight with the Naperian Case or the suspense he created and maintained until the final unveiling of Morgan's own theory at the very end. He gave the Terry lectures at Yale; the Vanuxem, at Princeton; the Leidy, at Pennsylvania, where he received the first award of the Leidy medal.

In 1931–32 he was Visiting Professor at Keio University, Tokio, and in '35–'36, Eastman Visiting Professor at Oxford. After retirement, he gave the Patten lectures at Indiana; became Visiting Professor for one year and remained as Research Associate at the University of California in Los Angeles.

Dr. Jennings was active on the Editorial Boards of four Journals; was a member of the National Academy, the American Philosophical Society and other coveted American Academies. He was President of the American Society of Zoologists (1909). His presidential address (1911) to the American Naturalists on Heredity and Personality—one of the most memorable of his gems—exposed, with a gaiety born of many insights, the genetic and environmental odds against the birth of any particular individual. Foreign recognitions included Honorary Fellowship in the Royal Microscopical Society, Corresponding Memberships in the Russian Academy of Sciences and in the Société Biologique de France or de Paris —he was not certain which. His honorary degrees proved quite unmanageable. The British Who's Who for 1944 records eight such degrees; the American counterpart and the American Men of Science both also in 1944 each list six. Agreement on totals, however, obscures the true diversities among four D.Sc.'s, three LL.D.'s and an Oxford A.M.; one D.Sc. and five LL.D.'s; and four D.Sc.'s with two LL.D.'s. One highly reputable LL.D. he avoided. As long as there were Paramecia to measure, count and keep in order, why bother about honorary degrees?

Whoever recalls him will continue to regret that his work at the Marine Biological Laboratory ended in 1933 and that he was a regular attendant only during the decade of the twenties. We should have liked to share him with many others. His inspiring example and achievement can be fully appreciated only against his background of almost continuous ill health. Yet he was always friendly and cheer-

ful; always excited about something; and ready to discuss a case, not for the sake of argument, but because of his passions for both sides of every question; for clarity of mind and for fairness. Impelled as he was by ceaseless cerebration, he nevertheless lists two related forms of recreation—travel and motoring. As a travelling companion, one can hardly imagine another more delightful; however, his friends who either rode with him or merely observed him spiraling briskly down the main street of Woods Hole were far too apprehensive to benefit from the recreative powers of his driving.

Samuel Ottmar Mast

By B. H. Willier

On October 5, 1871, Samuel Ottmar Mast was born on a farm near Ann Arbor, Michigan. His early schooling, academic training and teaching experience were in his native state. After obtaining a "full diploma" in 1897 from the Michigan State Normal College, he went to the University of Michigan where he received the B.S. degree in 1899. From 1899 to 1908, he was Professor of Botany and Biology at Hope College, Holland, Michigan. In 1906, he received the Ph.D. degree in zoology from Harvard University. He then came to the Eastern seaboard where he spent the remainder of his life. For a period of three years (1908–1911) he was a member of the biology staff at Goucher College. In the autumn of 1911, he joined the zoology staff at Johns Hopkins as associate professor which soon culminated in a Professorship of Zoology and later upon the retirement of the late Professor H. S. Jennings in the chairmanship of the Department of Zoology (1938–41). Since 1942, he has been professor emeritus of zoology.

According to the records, he first attended the Marine Biological Laboratory during the summer session of 1907. In 1908, he was elected a member of the Corporation and later (1936–1942) served as a member of the Board of Trustees and of its executive committee for two years (1938–1940). Since 1942 he has been Trustee Emeritus. He and his family have been regular summer residents of Woods Hole for a period of over twenty years. Over these years, he has been in regular attendance as an investigator and his interest in the laboratory has been constant and genuine.

In 1908, he married Grace Rebecca Tennent, the sister of the late David Hilt Tennent of Bryn Mawr and of this laboratory. She and three daughters and many grandchildren survive him. He died quite suddenly on Monday evening, February 3, 1947, at the age of 75 at his home in Roland Park in the city of Baltimore.

The life long work of Professor Mast was directed toward an understanding of the physiology of the "lower" organisms, especially the Protozoa. His major interest was in the mechanisms of behavior of these forms and more specifically in their reactions to light. This is best exemplified in his most significant book, "Light and the Behavior of Organisms" (1911) and in his numerous published papers on the motor responses, factors in the process of orientation, etc. of a variety of unicellular animals and other invertebrates. In 1926, as a result of his interest in the behavior of Amoeba proteus he formulated a theory to account for amoeboid movement, which has received wide recognition. His wide interest in the physiology of the Protozoa led him later to make a study of the nutrition of the colorless flagellate, Chilomonas. Together with Dr. Donald M. Pace and other students, he showed

that this organism in the total absence of light can synthetize carbohydrates, fats, proteins and protoplasm from a few simple inorganic salts, resembling in this respect, the green plants and certain bacteria. During the last few years of his life, Mast turned his attention to an investigation of the processes of feeding and digestion in the Protozoa, which culminated in his most significant paper on this subject entitled, "The Food-Vacuole of Paramecium." This work is a fitting and lasting example of the exactness and care which characterized all of his researches and publications. His every publication was marked by the meticulous care with which each word and phrase were weighed to make sure they meant exactly what he had in mind, no more and no less.

Professor Mast commanded to a marked degree the loyalty and admiration of graduate students. He had many pupils and has trained a whole generation of zoologists who have much to thank him for. His loss is deeply felt by many friends, former colleagues and students, and no less by the community of biologists at Woods Hole.

Dr. Lorrande Loss Woodruff

By R. G. Harrison

Lorande Loss Woodruff, Colgate Professor of Protozoology at Yale University and Director of the Osborn Zoological Laboratory, died at his home in New Haven after a long illness on June 23, 1947 in his 68th year. With his passing, the Corporation of the Marine Biological Laboratory loses a member of more than forty years standing and the Board of Trustees, one who had served faithfully for 24 years.

Dr. Woodruff was born in New York on July 14, 1879, and received his education in his native city, graduating from Columbia University with the A.B. degree in 1901 and the Ph.D. in 1905. Before completing his graduate work, he was appointed Assistant and later Instructor in Biology at Williams College, where he remained until he was called to Yale in 1907. There he served successively as Instructor, Assistant Professor and Professor, until his death. He became Chairman of the department and Director of the Osborn Zoological Laboratory in 1938, but took leave of absence in November 1946 on account of ill health.

His connection with the Marine Biological Laboratory began in 1905 when he attended the summer session as Investigator and Instructor in the Invertebrate Course and was elected to membership in the Corporation. Four years later, he joined the Staff of the course in Embryology of which he remained a member until 1914. During the absence of Dr. Calkins in the summer of 1927, he was in charge of the course in Protozoology. Elected to the Board of Trustees in 1923, he served with them until his death and during the years 1930–32, he was a member of the Executive Committee.

Coming to Yale at a time when a radical reorganization of the instruction in biology was to be undertaken, Woodruff took part from the first in teaching general biology and in 1910 assumed full charge of the general course in Yale College. This he built up into one of the soundest and at the same time, most popular courses in the University. Through the years, thousands of students listened to his masterly lectures, later incorporated in his textbook, "The Foundations of Biology," which has been very widely used and has run through six editions.

Woodruff's research was exclusively in the field of unicellular organisms. Beginning with his doctoral dissertation, which was done under the direction of the late Professor Calkins and published in 1905, he made many contributions to our knowledge of the life history of ciliates, their division rate, nuclear reorganization, the effect of environmental factors on their life cycle. He is perhaps best known for the famous pedigreed race of Paramecium aurelia, which was carried for eight years with daily isolation of the products of division, thus precluding conjugation and showing that these organisms can reproduce asexually indefinitely without dying out. In the first eight years over 5,000 generations were obtained and afterward the culture was carried in a less rigorous manner though sufficiently carefully to exclude conjugation except possibly between closely related individuals. The culture has now reached more than 24,000 generations without loss of vigor. In the course of this work, Woodruff and Erdmann discovered that, corresponding to the rhythms in division rate, the nuclei of the paramecia undergo a reorganization process which they termed "endomixis" and which they described as a form of nuclear reorganization with syncaryon formation. This stirred up much discussion and more recently the process has been described by others as autogamy, involving fusion between micronuclei from the same cell.

His research naturally attracted graduate students, and throughout the years many have written their dissertations under his direction and carried his methods to new centers, just as his assistants and students in the course in general biology, many of whom have become teachers, have spread his ideas of the teaching of biology throughout the land.

Woodruff was intensely interested in the history of science. Early in his career at Yale, he organized a course in the history of biology which he continued throughout his life. He was a collector of scientific books of historical significance. He published a number of essays and addresses in this field and organized two series of lectures on the history of science under the auspices of Gamma Alpha Fraternity, which were later published in book form under his editorship. A paper on "Baker on the Microscope and the Polypa" led to a friendly encounter with a descendant of Trembly, the famous author of the treatise on Hydra published in 1744.

Woodruff was Chairman of the Division of Biology and Agriculture of the National Research Council, 1928–29. He was a member of many scientific societies, including the National Academy of Sciences, the American Society of Zoologists, of which he was Secretary-Treasurer, 1907–09 and President in 1942, the American Physiological Society, the American Society of Naturalists (Vice-President, 1923), the American Association for the Advancement of Science, (Fellow) and others. He was a member of Phi Beta Kappa, Gamma Alpha and Sigma Xi, having been President of the Yale Chapter of the last in 1915. He lectured on Protozoology at four summer sessions of the Mountain Laboratory of the University of Virginia. For two terms of three years each, he was an Associate Editor of the *Journal of Morphology*. In 1935, he received the Townsend Harris medal from the College of the City of New York, where he had been as a student before entering Columbia.

For one who was closely associated with Woodruff for nearly forty years, it is difficult to realize that this intimate relation has been forever broken. The association was one of mutual trust throughout and without serious disagreement. He was always on the side of high standards, which he applied to himself as well as to

others. Indeed, this was one of his outstanding qualities, as was his intense loyalty to the institutions he served. He was quiet and reserved, but with a kindliness that meant much to those about him. With all of his reserve, he could be deeply moved, and he never recovered from the shock of Mrs. Woodruff's death, which came with such cruel suddenness in March 1946.

The members of the Corporation of the Marine Biological Laboratory desire to record their sorrow over the loss of one of their body, a friend and a fellow servant whom they will miss acutely and whose memory they will always cherish.

2. The Staff, 1947

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
*Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
Libbie H. Hyman, American Museum of Natural History.
A. C. Redfield, Woods Hole Oceanographic Institution.

II. Instructors

F. A. Brown, Associate Professor of Zoology, Northwestern University, in charge of course.
W. D. Burbanck, Professor of Biology, Drury College.
C. G. Goodchild, Professor of Biology, S.W. Missouri State College.
John H. Lochhead, Professor of Zoology, University of Vermont.
Madelene E. Pierce, Associate Professor of Zoology, Vassar College.
W. M. Reid, Associate Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.
T. H. Waterman, Instructor in Biology, Yale University.

III. Laboratory Assistants

Virginia L. Fogerson, Vassar College.
Amos L. Hopkins, Harvard University.
Marie Wilson, Western Maryland College.

EMBRYOLOGY

I. Consultants

P. B. Armstrong, Professor of Anatomy, College of Medicine, Syracuse University
H. B. Goodrich, Professor of Biology, Wesleyan University.

* Deceased.

II. Instructors

Donald P. Costello, Professor of Zoology, University of North Carolina, in charge of course.
Howard L. Hamilton, Assistant Professor of Zoology, Iowa State College.
John A. Moore, Assistant Professor of Zoology, Barnard College.
Jean Brachet, Professor of Experimental Morphology, University Brussels.

III. Research Assistant

Marjorie Hopkins Fox, University of California.

IV. Laboratory Assistants

Catherine Henley, The Johns Hopkins University.
Alice H. Ferguson.

PHYSIOLOGY

I. Consultants

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.

II. Instructors

Arthur K. Parpart, Associate Professor of Biology, Princeton Universtiy, in charge of course.
E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago.
Rudolph T. Kempton, Professor of Zoology, Vasser College (absent in 1946).
M. J. Kopac, Assistant Professor, New York University.
George Wald, Associate Professor of Biology, Harvard University.
Dorothy Wrinch, Lecturer, Smith College.

BOTANY

I. Consultants

S. C. Brooks, Professor of Zoology, University of California.
P. R. Burkholder, Eaton Professor of Botany, Yale University.
W. R. Taylor, University of Michigan.

II. Instructors

Maxwell S. Doty, Instructor of Botany, Northwestern University. In Charge of Course.
Hannah Croasdale, Dartmouth College.
Isabella Abbott, University of California.

III. Research Assistants

R. D. Northcraft, Massachusetts State College.
R. D. Wood, Northwestern University.

IV. Laboratory Assistant

Edwin T. Moul, University of Pennsylvania.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian
Deborah Lawrence, Assistant Librarian
Margaret P. McInnis — Mary A. Rohan
Jean Goodfellow

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham — Dorothy LeFevre

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist
Ruth Crowell — Marcia McLaughlin
M. B. Gray — W. E. Kahler — R. E. Tonks
A. M. Hilton — W. S. Landers — F. N. Whitman
G. Lehy

GENERAL OFFICE

F. M. MacNaught, Business Manager
Homer P. Smith, Assistant Business Manager
Polly L. Crowell — Mrs. Lila S. Myers

GENERAL MAINTENANCE

T. E. Larkin, Superintendent
Robert Adams — G. T. Nickelson, Jr.
R. W. Kahler — A. J. Pierce
Geo. Kahler — T. E. Tawell

THE GEORGE M. GRAY MUSEUM

3. Investigators and Students

Independent Investigators, 1947

Abbott, Isabella A., Graduate Student, University of California.
Abelson, Philip H., Chairman Bio Physics Section, Carnegie Inst. of Washington.
Allen, M. Jean, Instructor in Biology, Mather College, Western Reserve University.

ANDERSON, RUBERT S., Assistant Professor of Physiology, University of Maryland Medical School.
ARMSTRONG, PHILIP B., Professor of Anatomy, College of Medicine, Syracuse University.
ATLAS, MEYER, Assistant Professor of Biology, Yeshiva University.
BAEZ, SILVIO, Research Fellow, Cornell Medical College.
BALL, ERIC G., Professor of Biological Chemistry, Harvard Medical School.
BARRON, E. S. GUZMAN, Associate Professor of Biochemistry, The University of Chicago.
BARTLETT, JAMES H., Professor of Theoretical Physics, University of Illinois.
BARTON, ELEANOR, Assistant Professor of Zoology, N. J. College for Women, Rutgers University.
BERGER, CHARLES A., Director, Biological Laboratory, Fordham University.
BLISS, ALFRED F., Assistant Professor of Physiology, Tufts College Medical School.
BLUM, HAROLD F., Guggenheim Fellow, National Cancer Institute.
BOELL, E. J., Professor of Biology, Yale University.
BONNER, JOHN T., Junior Fellow, Harvard University.
BRACHET, JEAN L. A., Visiting Professor, University of Pennsylvania.
BRIDGMAN, JOSEPHINE, Associate Professor of Biology, Limestone College.
BRONK, DETLEV W., Professor of Biophysics and Director, Johnson Foundation, University of Pennsylvania.
BROWN, FRANK A., JR., Professor of Zoology, Northwestern University.
BROWNELL, KATHERINE A., Instructor, Ohio State University.
BRUST, MANFRED, Graduate Student, University of Chicago.
BUDINGTON, ROBERT A., Professor of Zoology Emeritus, Oberlin College.
BUNO, WASHINGTON, Professor and Director Department of Histology, School of Medicine, Montevideo.
BURBANCK, W. D., Professor of Biology, Drury College.
BURGER, ANDRE, Visitor at Harvard University.
CHAMBERS, FRANCIS W., JR., Lt. Com. U. S. Navy through Columbia University.
CHAMBERS, ROBERT, Research Professor of Biology, New York University.
CHASE, AURIN M., Assistant Professor of Biology, Princeton University.
CHENEY, RALPH H., Associate Professor of Biology, Brooklyn College.
CHRYSTALL, FRIEDA L., Teacher of Biology, New York City Public High School.
CLAFF, C. LLOYD, Research Fellow in Surgery, Harvard Medical School.
CLARK, A. M., Instructor in Biology, University of Delaware.
CLARK, ELIOT R., Professor of Anatomy, University of Pennsylvania School of Medicine.
CLARK, L. B., Professor of Biology and Chairman of Department, Union College.
CLEMENT, A. C., Professor of Biology, College of Charleston.
CLOWES, G. H. A., Research Director Emeritus, Eli Lilly & Company.
COLE, KENNETH S., Professor of Biophysics, University of Chicago.
COLWIN, ARTHUR L., Assistant Professor of Biology, Queens College.
CONKLIN, E. G., Professor Emeritus of Biology, Princeton University.
COOPER, KENNETH W., Associate Professor of Biology, Princeton University.
COPLEY, A. L., Research Associate, N. Y. U. Washington Square College.
COPELAND, D. E., Assistant Professor of Zoology, Brown University.
CORNMAN, IVOR, Research Fellow, Sloan-Kettering Institute.
COSTELLO, DONALD P., Professor of Zoology, University of North Carolina.
CROADALE, HANNAH T., Associate in Zoology, Dartmouth College.
CROWELL, SEARS, Associate Professor of Zoology, Miami University.
CURTIS, WINSTON C., Professor Emeritus of Zoology, University of Missouri.
DAN, JEAN C., Independent Investigator, Misaki Marine Biological Station, Japan.
DOTY, MAXWELL S., Assistant Professor of Botany, Northwestern University.
DUMM, MARY E., Instructor in Chemistry, New York University Medical College.
DURYEE, WILLIAM R., Guest Investigator, Carnegie Institute of Washington.
EDGERLEY, ROBERT H., Assistant Professor of Zoology, University of Alabama.
EICHEL, BERTRAM, Teaching Research Fellow, N. Y. U. College of Dentistry.
EVANS, TITUS C., Assistant Professor of Radiobiology, College of Physicians and Surgeons.
FAILLA, G., Professor of Radiology, College of Physicians and Surgeons.
FIGGE, FRANK H. J., Associate Professor of Anatomy, University of Maryland School of Medicine.

FISHER, HARVEY F., Western Reserve University.
FROEHLICH, ALFRED, Associate, May Institute for Medical Research.
GABRIEL, MORDECAI L., Instructor of Biology, Brooklyn College.
GILMAN, LAUREN C., Assistant Professor of Zoology, University of South Dakota.
GLASER, OTTO, Professor of Biology, Amherst College.
GOODCHILD, C. G., Professor of Biology, Missouri State College.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GORBMAN, AUBREY, Assistant Professor of Zoology, Columbia University.
GOULD, HARLEY N., Professor of Biology, Newcomb College.
GROSCH, DANIEL S., Assistant Professor, University of North Carolina.
GRUNDFEST, HARRY, Research Associate in Neurology, Columbia University Medical School.
HALL, THOMAS S., Associate Professor of Zoology, Washington University.
HAMILTON, HOWARD L., Assistant Professor of Zoology, Iowa State College.
HARTMAN, FRANK A., Professor of Physiology, Ohio State University.
HARVEY, ETHEL BROWNE, Research Biology Department, Princeton University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HEIDENTHAL, GERTRUDE, Assistant Professor of Biology, Russell Sage College.
HEILBRUNN, L. V., Professor of Zoology, University of Pennsylvania.
HIBBARD, HOPE, Professor of Zoology, Oberlin College.
HICKSON, ANNA KELTCH, Research Chemist, Eli Lilly & Company.
HINTON, TAYLOR, Instructor, Amherst College.
HOPKINS, HOYT S., Associate Professor of Physiology, N. Y. U. College of Dentistry.
HSIAO, SIDNEY C., Visitor and Seessel Fellow, Yale University.
HUTCHINGS, LOIS M., Instructor, N. Y. U. Washington Square College.
IFFT, JOHN D., Assistant Professor of Biology, Simmons College.
JABLONS, BENJAMIN, Associate Clinical Professor, New York Medical College.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JENKINS, GEORGE B., Emeritus Professor of Anatomy, George Washington University.
JEROME, SISTER FRANCIS, Professor of Biology, Ohio State University.
KARUSH, FRED, Research Associate, N. Y. U. Medical School.
KEMP, MARGARET, Associate Professor of Botany, Smith College.
KEMPTON, RUDOLPH T., Professor of Zoology, Vassar College.
KISCH, BRUNO, Professor at Yeshiva University.
KITCHEN, I. C., Research Fellow, Princeton University.
KLOTZ, IRVING M., Assistant Professor of Chemistry, Northwestern University.
KOPAC, M. J., Associate Professor of Biology, N. Y. U. Washington Square College.
KRAHL, M. E., Assistant Professor of Pharmacology, Washington University.
KREEZER, GEORGE L., Guggenheim Fellow, Princeton University.
KRUGELIS, EDITH J., Research Associate, University of Pennsylvania.
KUFFLER, STEPHEN W., Assistant Professor of Physiological Optics, Johns Hopkins Medical School.
LAVIN, GEORGE I., in charge of Spectroscopic Laboratory, Rockefeller Institute for Medical Research.
LAZAROW, ARNOLD, Assistant Professor of Anatomy, Western Reserve University.
LEFEVRE, PAUL G., Instructor in Physiology, University of Vermont.
LIBET, BENJAMIN, Instructor in Physiology, University of Chicago.
LIU, CHIEN-KANG, Graduate Student, McGill University.
LILLIE, RALPH S., Professor Emeritus of Physiology, University of Chicago.
LOCHHEAD, JOHN H., Assistant Professor of Zoology, University of Vermont.
LYNN, W. GARDNER, Associate Professor, The Catholic University of America.
MACDOUGALL, MARY S., Professor of Zoology, Agnes Scott College.
MARMONT, GEORGE, Assistant Professor of Biophysics, University of Chicago.
MARINELLI, L., Physicist, Memorial Hospital.
MARSHAK, ALFRED, Research Associate, New York University Medical College.
MARSLAND, DOUGLAS A., Associate Professor of Biology, N. Y. U. Washington Square College.
MAYOR, JAMES W., Research Professor of Biology, Union College.
MAZIA, DANIEL, Associate Professor of Zoology, University of Missouri.
MCDONALD, SISTER ELIZABETH SETON, Professor of Biology, College of Mt. St. Joseph.

MEMHARD, ALLEN R., Riverside, Connecticut.
METZ, CHARLES B., Assistant Professor of Zoology, Yale University.
METZ, C. W., Chairman, Department of Zoology, University of Pennsylvania.
MILLER, JAMES A., Chairman, Department of Anatomy, Emory University.
MOORE, JOHN A., Assistant Professor of Zoology, Barnard College.
MOUL, EDWIN T., Assistant Instructor of Botany, University of Pennsylvania.
NABRIT, S. M., Professor of Biology, Atlanta University and Moreland College.
NACHMANSOHN, DAVID, Research Associate in Neurology College of Physicians and Surgeons.
NIE, DASHU, Research Fellow, Institute of Zoology, Academia Sinica, China.
NORTHROP, JOHN H., Member of the Institute, Rockefeller Institute, Princeton.
O'BRIEN, JOHN A., JR., Assistant Professor of Biology, Catholic University of America.
OLMSTED, FREDERICK, Member Research Staff, Cleveland Clinic.
OSTERHOUT, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
PAPPENHEIMER, A. M., Associate Professor of Bacteriology, New York University.
PARMENTER, CHARLES L., Professor of Zoology, University of Pennsylvania.
PARPART, ARTHUR K., Professor of Biology, Princeton University.
PATT, DONALD I., Instructor in Biology, Middlebury College.
PEQUEGNAT, WILLIS E., Assistant Professor of Zoology, Pomona College.
PIERCE, MADELENE E., Associate Professor, Vassar College.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
PROSSER, C. LADD, Associate Professor of Zoology, University of Illinois.
REID, W. MALCOLM, Associate Professor and Department Head of Biology, Monmouth College.
ROBBIE, WILBUR A., Research Assistant Professor, State University of Iowa.
ROGICK, MARY D., Professor of Biology, College of New Rochelle.
ROOFE, PAUL G., Professor of Anatomy, University of Kansas.
ROTH, ALEXANDER, Research Assistant, University of Kansas.
RUDZINSKI, ANNA MARIA, Research Worker, Washington Square College.
RUGH, ROBERTS, Associate Professor of Biology, N. Y. U. Washington Square College.
RULON, OLIN, Assistant Professor of Biology, Wayne University.
SCHMIDT, GERHART, Research Fellow, Tufts Medical School.
SCHNEYER, LEON H., Instructor, New York University Dental College.
SCOTT, ALLAN, Associate Professor of Biology, Union College.
SHANES, ABRAHAM M., Assistant Professor of Physiology, New York University College of Dentistry.
SHAPIRO, HERBERT, Physiologist, National Institute of Health.
SHERMAN, FREDERICK G., Instructor of Biology, Brown University.
SICHEL, F., Professor of Physiology, University of Vermont.
SLIFER, ELEANOR H., Assistant Professor of Zoology, State University of Iowa.
SMITH, SYDNEY, University Lecturer, Cambridge University, England.
STEWART, DOROTHY R., Assistant Professor of Anatomy, Western Reserve Medical School.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STEINBACH, H. B., Professor of Zoology, University of Minnesota.
STOKEY, ALMA G., Professor Emeritus, Mount Holyoke College.
STOUDT, HARRY N., Instructor in Biology, Temple University.
STRAUS, WILLIAM L., Associate Professor of Anatomy, Johns Hopkins University.
STUNKARD, HORACE W., Professor of Biology, New York University.
SZENT-GYORGYI, ALBERT, Professor of Biochemistry, Budapest University, Budapest.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan.
TOWNSEND, GRACE G., Professor of Biological Science, Cincinnati College of Pharmacy.
TRACY, HENRY, Professor of Anatomy, University of Kansas.
TREITEL, OTTO, Research Associate, Botanical Laboratory, University of Pennsylvania.
VILLEE, CLAUDE A., Instructor in Biological Chemistry, Harvard Medical School.
WAINIO, WALTER W., Assistant Professor, New York University College of Dentistry.
WALD, GEORGE, Associate Professor, Harvard University.
WARD, HELEN L., Instructor in Zoology, University of Tennessee.
WARNER, ROBERT C., Assistant Professor of Chemistry, New York University College of Medicine.
WATERMAN, TALBOT, Instructor, Yale University.

WATTERSON, RAY L., Assistant Professor of Zoology, University of Chicago.
WHITING, ANNA R., Guest Investigator, University of Pennsylvania.
WHITING, P. W., Professor of Zoology (Genetics), University of Pennsylvania.
WICHTERMAN, RALPH, Associate Professor of Biology, Temple University.
WILBER, CHARLES G., Assistant Professor of Physiology, Fordham University.
WILDE, C. E., JR., Instructor of Biology, Princeton University.
WINSOR, CHARLES P., Assistant Professor, School of Hygiene, Johns Hopkins University.
WITKUS, ELEANOR R., Instructor in Botany and Cytology, Fordham University.
WRINCH, DOROTHY, Lecturer, Smith College.
YNTEMA, CHESTER L., Associate Professor of Anatomy, Syracuse University Medical College.
ZINN, DONALD J., Instructor in Zoology, Rhode Island State College.
ZORZOLI, ANITA, Instructor in Physiology and Biochemistry, Washington University.
ZWEIFACH, BENJAMIN W., Assistant Professor of Physiology, Cornell Medical College.

Beginning Investigators, 1947

ALSCHER, RUTH P., Instructor in Biology, Manhattanville College.
ALLEN, MARY BELLE, Research Fellow, Washington University.
BERG, GEORGE G., Graduate Student, Columbia University.
BLUMENTHAL, GERTRUDE, University of Pennsylvania.
BRONFENBRENNER, ALICE, Medical Student, Washington University Medical School.
CRANE, ROBERT K., Graduate Student, Harvard University.
FAHEY, ELIZABETH M., Boston University.
FERGUSON, ALICE, Graduate Assistant, Louisiana State University.
FOGERSON, VIRGINIA L., Assistant in Zoology, Vassar College.
FONTANELLA, M. A., Instructor in Comparative Anatomy, Fordham University.
FOWLE, ANN M. C., Research Assistant, University of Toronto.
GOLLUB, SEYMOUR, Student, University of Pennsylvania.
GREEN, JAMES W., Graduate Student, Princeton University.
HERBRUCK, BRUCE K., Student, Western Reserve University School of Medicine.
KUNTZ, ELOISE, Assistant in Biology, Brown University.
LERNER, ELEANOR, Fellow in Zoology, Washington University.
LOVELACE, ROBERTA, Teaching Fellow, University of North Carolina.
LUMB, ETHEL SUE, Graduate Assistant in Zoology, Washington University.
MARTIN, BARBARA A., Assistant in Zoology, Barnard College.
MCLEAN, DOROTHY J., Demonstrator, University of Toronto.
NELSON, THOMAS CLIFFORD, Graduate Assistant, Columbia University.
NURNBERG, MIRIAM, Graduate Student, New York University.
PETTENGILL, OLIVE S., Graduate Assistant in Physiology, Brown University.
RANSMEIER, ROBERT E., Graduate Student, University of Chicago.
RECKNAGEL, RICHARD O., Student, University of Pennsylvania.
SCHLESINGER, ARTHUR H., Research Fellow, Northwestern University.
SHAVER, JOHN R., Instructor in Zoology, University of Pennsylvania.
SIROT, GUSTAVE, Western Reserve University.
TAYLOR, BABETTE, Graduate Student, University of Minnesota.
TIETZE, F., Research Fellow, Northwestern University.
VAN HOESEN, Drusilla, Graduate Student, University of Pennsylvania.
WEINER, MILTON H., Student, Western Reserve University.
WEINSTEIN, H. G., Research Assistant, University of Illinois.
WILSON, JEAN R., Graduate Student, University of Pennsylvania.
WILSON, WALTER L., Assistant Instructor in Zoology, University of Pennsylvania.
WITTENBERG, JONATHAN, Student, Columbia University.

Research Assistants, 1947

ABRAMSKY, TESS, Research Assistant, Rockefeller Institute for Medical Research.
BENSON, ELEANORE, Research Assistant, University of Pennsylvania.
BERMAN, JACK H., Graduate Student, Western Reserve University.
BERMAN, RUTH, Student and Research Assistant, University of Pennsylvania.
CARLSON, FRANCIS D., Research Assistant, Johnson Foundation, University of Pennsylvania.

CLIPPINGER, F. W., Student, Drury College.
CONNELLY, C. M., Graduate Student, Johnson Foundation, University of Pennsylvania.
COOPER, OCTAVIA, Research Assistant, Harvard Medical School.
COOPERSTEIN, SHERWIN, Student, New York University College of Dentistry.
DANUFSKY, PHILIP, Research Assistant, University of Pennsylvania.
DEY, THOMAS E., Research Technician, Princeton University.
DIETZ, ALMA, Student, University of Michigan.
ESTABLE, JOSE J., Professor of Pharmacology, University of Montevideo, Uruguay.
FERREIRA, HISS M., Fellow in Biophysics, University of Chicago.
FOLEY, MARY T., Research Assistant, Yale University.
FOX, MARJORIE HOPKINS, Instructor, San Francisco Junior College.
GOLD, MARCIA, Research Assistant, University of Chicago.
HENDEE, EDELMIRA D., Research Assistant, New York University Medical School.
HENLEY, CATHERINE, Research Assistant, University of North Carolina.
HIRSCHHORN, HENRY A., Student, New York University.
HOARE, CATHERINE V., Graduate Assistant in Bacteriology, Brown University.
HONEGGER, CAROL M., Instructor, Temple University.
JAFFE, LIONEL F., Harvard University.
KEMP, GRACE, Graduate Assistant, Wesleyan University.
METZ, DELILAH B., Research Associate in Medicine, Cornell Medical School.
MEZGER, LISELOTTE, Washington University.
MITCHELL, CONSTANCE, Research Assistant, University of Pennsylvania.
NEFF, ROBERT J., Research Assistant, University of Missouri.
NORRIS, KARL H., Electronic Engineer, University of Chicago.
NORTHCRAFT, RICHARD D., Instructor, Rutgers University.
PORTIS, RICHARD A., Graduate Student, Western Reserve University.
RALL, WILFRID, Fellow in Biophysics, University of Chicago.
RIESER, PETER, Research Assistant, University of Pennsylvania.
ROTHENBERG, M. A., Research Assistant in Neurology, Columbia University.
SANDEEN, MURIEL, Teaching Assistant, Northwestern University.
SEAMAN, GERALD R., Graduate Assistant, Fordham University.
VOLKMAN, ALVIN, Graduate, Union College.
WALTERS, C. PATRICIA, Research Assistant, Eli Lilly & Company.
WEBB, MARGUERITE, Teaching Assistant, Northwestern University.
WEISS, MICHAEL S., Research Assistant in Neurology, College of Physicians and Surgeons.
WHEELER, CHARLES B., Research Assistant, Anatomy Department, University of Kansas.
WILLIS, MARION, Research Assistant, University of Pennsylvania.
WILSON, MARIE, Assistant in Zoology, Northwestern University.
WOODWARD, ARTHUR A., JR., Instructor in Zoology, University of Pennsylvania.
YOUNG, MARCIA A., Technical Assistant, Ohio State University.

Library Readers, 1947

BERG, WILLIAM E., Research Fellow in Medical Physics, University of California.
BERNHEIMER, ALLAN W., Assistant Professor of Bacteriology, New York University College of Medicine.
BLOCH, ROBERT, Research Associate in Botany, Yale University.
CANTONI, G. L., Assistant Professor in Pharmacology, Long Island College of Medicine.
CLIFFORD, SISTER ADELE, Teacher, College of Mount St. Joseph.
DEANE, HELEN W., Instructor, Harvard Medical School.
FRIES, E. F. B., Assistant Professor of Biology, City College of New York.
GATES, R. RUGGLES, Research Fellow in Biology, Harvard University.
GRANT, MADELEINE, Member Teaching Faculty, Sarah Lawrence College.
GUDERNATSCH, FREDERICK, Visiting Professor, New York University.
GUREWICH, VLADIMIR, Assistant Visiting Physician, Bellevue Hospital.
HARRISON, JOHN W., Student, Medical School, University of Pennsylvania.
HOBSON, LAWRENCE B., Assistant. Resident, New York Hospital.

JOFTES, DAVID L., Assistant in Biology, Tufts College.
JONASSEN, HANS B., Assistant Professor of Chemistry, Tulane University.
LANGE, MATHILDE M., Professor of Zoology, Wheaton College.
LEDERBERG, JOSHUA, Fellow, Jane Coffin Childs Fund, Yale University.
LEVITT, LEO C., Graduate Student in Physics, Princeton University.
LEVEY, STANLEY, Instructor in Biochemistry, Wayne University College of Medicine.
LOEWI, OTTO, Research Professor of Pharmacology, New York University College of Medicine.
MEYERSHOF, OTTO, Research Professor of Biochemistry, University of Pennsylvania.
MOUTON, ROBERT F., Head of Mission, Belgium.
OSTER, ROBERT H., Associate Professor of Physiology, University of Maryland School of Medicine.
PICK, JOSEPH, Associate Professor of Anatomy, New York University College of Medicine.
PRICE, BRONSON, Analyst, U. S. Public Health Service.
ROSE, S. MERYL, Assistant Professor, Smith College.
RYAN, FRANCIS J., Assistant Professor of Zoology, Columbia University.
SCHNEIDER, LILLIAN K., Research Assistant in Microbiology, Columbia University.
SCHWARTZMAN, GREGORY, Head of Department of Bacteriology, Mt. Sinai Hospital.
SIEGEL, BENJAMIN, Associate in Laboratory of Electron Microscopy, Polytechnic Institute.
SPRATT, NELSON T., JR., Assistant Professor of Biology, Johns Hopkins University.
TAUSSKY, HERTHA H., Research Associate, Cornell University Medical College.
VONSALLMAN, LUDWIG, Associate Professor, College of Physicians and Surgeons.
WAGMAN, IRVING H., Associate in Physiology, Jefferson Medical College.
WATTS, NELLIE P., Pharmacologist, Abbott Laboratories.
ZAWADZKI, BRONISLAW, Fellow, College of Physicians and Surgeons.

Students, 1947

BOTANY

BOYLE, E. MARIE, Science Teacher, Baldwin School.
CADORET, REMI, Student, Harvard College.
COYLE, ELIZABETH E., Assistant Professor of Biology, College of Wooster.
DEARDEN, ELIZABETH R., Research Assistant, University of Toronto.
DIPPELL, RUTH D., Research Associate, Indiana University.
DWORKIN, ZELMAN Z., Instructor, University of Connecticut.
ERSKINE, DAVID S., Acadia University.
FERGUSON, EDWARD L., Undergraduate, Wesleyan University.
GAGE, MARILYN A., Student, Pennsylvania College for Women.
GRIMM, MADELON R., Research Assistant in Bacteriology, Amherst College.
HOLMES, ROBERT W., Student, Haverford College.
HULBURT, EDWARD M., Graduate Student, University of Michigan.
HYDE, BEAL B., Student, Harvard University.
LAWSON, DOROTHY L., Wellesley College.
SPIEGEL, LEONARD E., Drew University.
WOOD, RICHARD D., Northwestern University.
WOOD, URDA K., Northwestern University.

EMBRYOLOGY

BAUER, MARK H., Graduate Student, Princeton University.
BLAIR, CHARLES B., Graduate Student, Instructor, University of North Carolina.
BOLTON, ELLIS T., Graduate Assistant, Rutgers University.
BUCHANAN, DOUGLAS, Assistant in Biochemistry, Dartmouth Medical School.
CALVERT, JULIE N., Demonstrator in Biology, Bryn Mawr College.
CHMIELOWSKI, ADAM A., Graduate Assistant, Marquette University.
CLOUD, PRESTON E., JR., Assistant Professor of Geology, Harvard University.
CORTELYOU, REV. J. R., Student, Northwestern University.

DICKASON, MARY E., Smith College.
EDWARDS, JOHN P., Graduate, Drury College.
GOMBERG, CHARLES, Student, McGill University.
GREGG, JAMES H., Graduate Student, Princeton University.
HILL, HENRIETTA J., Dickinson College.
HINCHEY, M. CATHERINE, Instructor in Biology, Temple University.
HOLTZER, HOWARD, Student, University of Chicago.
HOPKINS, AMOS L., Harvard College.
ISAAC, ISAAC B., Wesleyan University.
KLAU, HELENE H., Graduate Assistant, University of Oklahoma.
LEONE, CHARLES A., Instructor, Rutgers University.
MAGDEBARGER, ALICE E., Student, Goucher College.
MAXON, MARION G., Graduate Assistant, Claremont Graduate School.
NELSON, BETTY G., Graduate Student, Johns Hopkins University.
ODUM, HOWARD T., Graduate Student, University of North Carolina.
PADYKULA, HELEN A., Graduate Assistant, Mt. Holyoke College.
RIGGS, AUSTIN F., Student, Harvard College.
ROBBINS, MARILYN, Student, Stanford University.
ROSENBLOOM, LIBBY, Laboratory Assistant, University of Michigan.
RUSSO, EVELYN E., Rosemont College.
SHRADER, RUTH E., Graduate Student, Yale University Medical School.
STOLACK, RICHARD B., Graduate Assistant, University of North Carolina.
WEINSTEIN, HYMAN G., Research Assistant, University of Chicago.
WENGER, BYRON S., Graduate Assistant, Washington University.
ZUCKERKANDL, EMIL, University of Illinois.

PHYSIOLOGY

ARDAO, MARIA I., Assistant Professor of Chemistry, Montevideo, Uruguay.
BATEMAN, MARGARET M., Graduate Student, Washington University.
BICKS, RICHARD O., Long Island College of Medicine.
BLOCH, SAMUEL W., Hammond, Louisiana.
COSGROVE, WILLIAM B., Graduate Assistant, New York University.
DAS, S. M., Government Scholar, Government of India.
EDELBERG, ROBERT E., Graduate Student, University of Pennsylvania.
FIALA, SILVIO, Charles University, Prague.
FRIEDMAN, FLORENCE L., Teaching Assistant, Washington University.
GOURLEY, D. R. H., Research Assistant, University of Toronto.
HAMILTON, JAMES D., Fellow, Department of Medical Research, University of Western Ontario.
HANKE, HARRIETT, Teaching Fellow in Biology, New York University.
IRVING, JACK HOWARD, Graduate Student, Princeton University.
KAUPE, WALTER, Massachusetts Institute of Technology.
KELLOGG, RALPH H., Teaching Fellow in Physiology, Harvard Medical School.
LAYTON, LAURENCE L., Assistant Professor, Johns Hopkins University.
LOVE, WARNER E., Graduate Student, University of Pennsylvania.
MCCANN, WILLIAM P., Cornell University Medical College.
MOSKOVIC, SAMUEL, Graduate School, New York University.
NELSON, LEONARD, Graduate Teaching Assistant, Washington University.
PAULSEN, ELIZABETH C., Instructor in Zoology, University of Vermont.
PERLMAN, PRESTON L., Research Fellow, Cornell University.
RANSMIER, ROBERT E., Graduate Student, University of Chicago.
STEELE, ALLOYS C., Research Fellow, University of Toronto.
STEKLER, BURTON, Student, New York University Medical School.
TAYLOR, ROBERT E., Fellow in Physiology, Strong Memorial Hospital.

INVERTEBRATE ZOOLOGY

ALLEN, JOHN M., Drury College.
ALSCHER, RUTH P., Instructor in Biology, Manhattanville College.
ANDERTON, LAURA G., Laboratory Assistant, Brown University.
BAUER, EDWARD CHARLES, Undergraduate Assistant, University of Connecticut.
BOSS, WILLIS R., University of Iowa.
BOYER, GEORGE F., Graduate Student, University of Illinois.
BRAGG, NANA I., Student, Oberlin College.
BUCKLIN, DONALD H., Graduate Assistant, Amherst College.
BULL, ALICE L., Laboratory Assistant, Mt. Holyoke College.
BURGER, ANDRE, Harvard University.
CHAMBERLIN, J. LOCKWOOD, Tufts College.
CLOUD, PRESTON E., Assistant Professor, Harvard University.
COLE, GERALD A., Laboratory Assistant, University of Minnesota.
CORLISS, CLARK E., Graduate Student, University of Massachusetts.
DANIEL, EDWIN E., Student, Johns Hopkins University.
EHRLICH, MIRIAM, Graduate Student, Yale University.
EPSTEIN, HOWARD E., Assistant, Department of Comparative Anatomy, Cornell University.
EVANS, JEANNE F., Student, University of Pennsylvania.
FLYNN, JOYCE M., Newton, Massachusetts.
FORD, BENJAMIN P., Princeton University.
FORD, DONALD H., Laboratory Assistant, Wesleyan University.
FORD, PRISCILLA W., Wellesley College.
FULLER, FORST D., Instructor in Zoology, Purdue University.
GLOCKLER, ANNABEL, Western Maryland College.
GOODSMITH, WILLOUGHBY, Teaching Assistant and Student, Amherst College.
HAY, ELIZABETH D., Undergraduate, Smith College.
HENOCH, STEPHANIE D., Graduate Assistant, Indiana University.
HOWARD, ROBERT S., University of Chicago.
HUTCHINGS, LOIS M., Instructor, N. Y. U. Washington Square College.
KAMNER, SANDRA L., Goucher College.
KELLER, MILDRED E., Randolph-Macon Woman's College.
MANDLOWITZ, SAMUEL, Tulane University.
MCCULLOUGH, KIRK W., Instructor, Washington and Jefferson College.
MCELLIGOTT, JANE K., Graduate Student, Fordham University.
MCGUIRE, IRENE E., Fordham University.
MCWHINNIE, MARY A., Instructor in Zoology, DePaul University.
MELTZER, JAY, Student, Princeton University.
ODUM, HOWARD T., Graduate Student, University of North Carolina.
PERNA, VINCENT, Student, Wesleyan University.
PRONKO, ROBERT C., Student, Drury College.
RADFORD, BETTY J., Assistant in Biology Department, Agnes Scott College.
SAUSE, GLADYS E., Western Maryland College.
SPRINGER, ELEANOR V., Vassar College.
STAY, BARBARA A., Student, Vassar College.
STEEVES, TAYLOR A., East Weymouth, University of Massachusetts.
STOLACK, RICHARD B., Graduate Student, University of North Carolina.
SUSCA, LOUIS, Graduate Student, Fordham University.
THAYER, PHILIP S., Amherst College.
TRENT, JANE, Assistant, Wesleyan University.
WENGER, BYRON S., Graduate Assistant, Washington University.
WINN, HUDSON S., Graduate Teaching Assistant, Northwestern University.
WINSTON, PAUL W., Student, University of Massachusetts.
WITHROW, JOANNA E. F., Student, Oberlin College.
WOLF, DORIS E., Graduate Student, University of Minnesota.
WONG, AN CHI, Wellesley College.

4. Tabular View of Attendance

	1943	*1944*	*1945*	*1946*	*1947*
Investigators—Total	160	193	212	267	299
Independent	89	112	138	175	182
Under instruction	19	11	10	29	36
Library readers	35	50	38	38	36
Research assistants	17	20	26	25	45
Students—Total	68	75	96	122	131
Zoology	47	37	55	57	55
Embryology	13	23	23	30	33
Physiology	8	10	13	26	26
Botany	—	5	5	9	17
Total Attendance	228	276	308	389	430
Less persons registered as both students and investigators	6	1			2
	222	275			428
Institutions Represented—Total	116	106	124	141	
By investigators	70	74	100	102	114
By students	41	41	49	56	56
Schools and Academies Represented					
By investigators	2	1	2	2	1
By students	1	2	2	—	1
Foreign Institutions Represented					
By investigators	2	2	1	7	7
By students	—	3	—	5	3

5. Subscribing and Cooperating Institutions, 1947

Cooperating Institutions

American Philosophical Society (Penrose Fund)
Amherst College
Brooklyn College
Brown University
Bryn Mawr College
The Catholic University of America
College of Mt. St. Joseph on the Ohio
Columbia University
Cornell University
Duke University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins Medical School
Johns Hopkins University
Johnson Foundation
Eli Lilly & Company
Macy Foundation
Massachusetts Institute of Technology
Memorial Hospital
Miami University
New York University
New York University College of Medicine
New York University School of Dentistry
New York University Washington Square College
Oberlin College
Ohio State University
Pomona College
Princeton University
Rockefeller Institute of Medical Research
State University of Iowa
Syracuse University Medical School
Temple University
Tufts College
Union College
University of Chicago
University of Illinois
University of Kansas
University of Maryland Medical School
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester
University of Toronto
University of Vermont
University of Virginia
Vassar College
Washington University
Wayne University
Wellesley College
Wesleyan University
Western Reserve Medical School
Wilson College
Woods Hole Oceanographic Institution
Yale University

Subscribing Institutions

Atlanta University, Moreland College
Boston Dispensary
Carnegie Inst. of Washington
College of Charleston
College of Physicians and Surgeons
Indiana University
Mount Sinai Hospital
National Cancer Institute
North Carolina State College of Agriculture & Engineering
Pennsylvania College for Women
Rockefeller Foundation
Russell Sage College
Rutgers University
Simmons College
Smith College
St. John's College
University of Delaware
University of Massachusetts
University of Michigan
University of Minnesota
University of Western Ontario

6. The Friday Evening Lectures, 1947

June 27
Dr. Gottfried Fraenkel ..... "Nutritional Research with Insects."
July 3
Dr. Jean Brachet ..... "Metabolism of Nucleic Acids in Embryonic Development."
July 11
Prof. E. L. Tatum ..... "Mutation in Microorganisms."
July 25
Dr. F. O. Schmitt ..... "Studies of the Ultra Structure of Connective Tissue Constituents."
August 1
Dr. Philip H. Abelson ..... "Tracer Isotopes in Biology."
August 8
Prof. Alfred S. Romer ..... In celebration of the 74th Anniversary of the founding of the Agassiz Laboratory at Penikese.
August 15
Dr. John A. Moore ..... "Early Development of Amphibian Hybrids."
August 22
Dr. Daniel Mazia ..... "The Molecular Structure of Chromosomes."

Other Lectures

July 24
Dr. Paul S. Galtsoff ..... "The Bikini Atom Bomb Test."
July 30
Dr. K. J. Heinicke ..... "Recent Developments in Microscopy."
August 13
Dr. Oscar W. Richards ..... "Phase Microscopy, with Special Reference to Biology."
August 18
Dr. H. J. Abraham ..... U. S. State Dept. Associate Director of Relations with UNESCO.
August 27
Dr. Albert Szent-Gyorgyi ..... "Muscular Contraction."
August 28
Prof. Louis van den Berghe ..... "National Parks and Scientific Research in the Congo."

7. Seminars, 1947

July 15

Anna R. Whiting ..... "Androgenetic Males from Eggs X-rayed with Dose Many Times Lethal."

T. S. Hall and Florence Moog ..... "Effects of Sodium Azide in Solution upon the Rate of Amphibian Development."

C. A. Villee and H. B. Bissell ..... "Nucleic Acids and Nucleotides as Growth Factors in Drosophila."

July 22

Ivor Cornman ..... "The Effects of Podophyllin on the Maturation and Cleavage of the Starfish Egg."

C. G. Wilber ..... "The Synthesis of Lipids from Protein in Colpidium Campylum."

W. W. Wainio, S. Cooperstein, S. Kollen and B. Eichel ..... "The Preparation of a Soluble Cytochrome Oxidase."

July 29

Otto Myerhoff and Jean R. Wilson .. "Glycolysis of Glucose, Fructose, and Hexosephosphates in Tumor and Brain Extracts."

B. Libet ..... "Relatively Steady Potentials and Brain Activity."

J. T. Bonner ..... "Morphogenetic Movement in the Slime Molds."

August 5

Edith J. Krugelis ..... "Alkalin Phosphatase Localization in Early Embryonic Development."

J. R. Shaver ..... "Experimental Study of the 'Second Factor' in Artificial Parthenogenesis in Frog Eggs."

Robert Bloch ..... "Irreversible Differentiation in Certain Plant Cell Lineages."

August 12

Dorothy Wrinch ..... "Biological Specificity and Biological Morphology."

Taylor Hinton ..... "Factors Influencing the Expression of 'Position Effects.'"

D. E. Copeland ..... "The Cytological Basis of Salt Excretion from the Gills of Fundulus heteroclitus."

August 19

P. W. Whiting ..... "Spermatogenesis in Sphecoid Wasps."

Ethel Browne Harvey ..... "Bermuda Sea Urchins and Their Eggs."

Paul S. Galtsoff ..... "The Red Tide along the Gulf Coast and Florida."

8. Members of the Corporation, 1947

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Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.

Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.

Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.

* Deceased.

CONKLIN, PROF. EDWIN G., Princeton University, Princeton, New Jersey.
COWDRY, DR. E. V., Washington University, St. Louis, Missouri.
JACKSON, MR. CHAS. C., 24 Congress Street, Boston, Massachusetts.
JACKSON, MISS M. C., 88 Marlboro Street, Boston, Massachusetts.
KING, MR. CHAS. A.
KINGSBURY, PROF. B. F., Cornell University, Ithaca, New York.
LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Maryland.
MEANS, DR. J. H., 15 Chestnut Street, Boston, Massachusetts.
MOORE, DR. GEORGE T., Missouri Botanical Gardens, St. Louis, Missouri.
MOORE, DR. J. PERCY, University of Pennsylvania, Philadelphia, Pa.
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SCOTT, DR. ERNEST L., Columbia University, New York City, New York.
SEARS, DR. HENRY F., 86 Beacon Street, Boston, Massachusetts.
SHEDD, MR. E. A.
STRONG, DR. O. S., Columbia University, New York City, New York.
WAITE, PROF. F. C., 144 Locust Street, Dover, New Hampshire.
WALLACE, LOUISE B., 359 Lytton Avenue, Palo Alto, California.

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ADAMS, DR. A. ELIZABETH, Mount Holyoke College, South Hadley, Massachusetts.
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ADOLPH, DR. EDWARD F., University of Rochester Medical School, Rochester, New York.
ALBAUM, DR. HARRY G., Biology Dept., Brooklyn College, Brooklyn, N. Y.
ALBERT, DR. ALEXANDER, Mayo Clinic, Rochester, Minnesota.
ALLEE, DR. W. C., The University of Chicago, Chicago, Illinois.
AMBERSON, DR. WILLIAM R., Department of Physiology, University of Maryland, School of Medicine.
ANDERSON, DR. RUBERT S., University of Maryland School of Medicine, Department of Physiology, Baltimore, Maryland.
ANDERSON, DR. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.
ANGERER, PROF. C. A., Department of Physiology, Ohio State College, Columbus, Ohio.
ARMSTRONG, DR. PHILIP B., College of Medicine, Syracuse University, Syracuse, New York.
AUSTIN, DR. MARY L., Wellesley College, Wellesley, Massachusetts.
BAITSELL, DR. GEORGE A., Yale University, New Haven, Connecticut.
BAKER, DR. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
BALLARD, DR. WILLIAM W., Dartmouth College, Hanover, New Hampshire.
BALLENTINE, DR. ROBERT, Columbia University, Department of Zoology, New York City, New York.
BALL, DR. ERIC G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.

BARD, PROF. PHILIP, Johns Hopkins Medical School, Baltimore, Maryland.
BARRON, DR. E. S. GUZMAN, Department of Medicine, The University of Chicago, Chicago, Illinois.
BARTH, DR. L. G., Department of Zoology, Columbia University, New York City, New York.
BARTLETT, DR. JAMES H., Department of Physics, University of Illinois, Urbana, Illinois.
BEADLE, DR. G. W., School of Biological Sciences, Stanford University, California.
BEAMS, DR. HAROLD W., Department of Zoology, State University of Iowa, Iowa City, Iowa.
BECK, DR. L. V., Edgey Road and Beech Avenue, Bethesda, Maryland.
BEERS, C. D., University of North Carolina, Chapel Hill, North Carolina.
BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, Louisiana.
BERTHOLF, DR. LLOYD M., Western Maryland College, Westminster, Maryland.
BEVELANDER, DR. GERRIT, New York University School of Medicine, New York City, New York.
BIGELOW, DR. H. B., Museum of Comparative Zoology, Cambridge, Massachusetts.
BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.
BISSONNETTE, DR. T. HUME, Trinity College, Hartford, Connecticut.
BLANCHARD, PROF. K. C., Johns Hopkins Medical School, Baltimore, Maryland.
BLUM, DR. HAROLD F., Department of Biology, Princeton University, Princeton, New Jersey.
BODINE, DR. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.
BOELL, DR. EDGAR J., Yale University, New Haven, Connecticut.
BORING, DR. ALICE M., Yenching University, Peiping, China.
BRADLEY, PROF. HAROLD C., University of Wisconsin, Madison, Wisconsin.
BRODIE, MR. DONALD M., 522 Fifth Avenue, New York City, New York.
BRONFENBRENNER, DR. JACQUES J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.
BRONK, DR. DETLEV W., Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania.
BROOKS, DR. MATILDA M., University of California, Department of Zoology, Berkeley, California.
BROOKS, DR. S. C., University of California, Berkeley, California.
BROWN, DR. DUGALD E. S., Bermuda Biological Station, Bermuda.
BROWN, DR. FRANK A., JR., Department of Zoology, Northwestern University, Evanston, Illinois.
BROWNELL, DR. KATHERINE A., Ohio State University, Columbus, Ohio.
BUCK, DR. JOHN B., Industrial Hygiene Research Lab., National Institute of Health, Bethesda, Maryland.
BUCKINGHAM, MISS EDITH N., Sudbury, Massachusetts.
BUDINGTON, PROF. R. A., Winter Park, Florida.
BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Virginia.
BULLOCK, DR. T. H., University of California, Los Angeles 24, California.
BURBANCK, DR. WILLIAM D., Department of Biology, Drury College, Springfield, Missouri.

BURKENROAD, DR. M. D., Yale University, New Haven, Connecticut.
BURKHOLDER, DR. PAUL R., Yale University, New Haven, Connecticut.
BUTLER, DR. E. G., Princeton University, Princeton, N. J.
CAMERON, DR. J. A., Baylor College of Dentistry, Dallas, Texas.
CANNAN, PROF. R. K., New York University College of Medicine, New York City, New York.
CARLSON, PROF. A. J., Department of Physiology, The University of Chicago, Chicago, Illinois.
CAROTHERS, DR. E. ELEANOR, 134 Avenue C. East, Kingman, Kansas.
CARPENTER, DR. RUSSELL L., Tufts College, Tufts College, Massachusetts.
CARROLL, PROF. MITCHELL, Franklin and Marshall College, Lancaster, Pennsylvania.
CARVER, PROF. GAIL L., Mercer University, Macon, Georgia.
CATTELL, DR. MCKEEN, Cornell University Medical College, New York City, New York.
CATTELL, MR. WARE, Cosmos Club, Washington, D. C.
CHAMBERS, DR. ROBERT, Woods Hole, Massachusetts.
CHASE, DR. AURIN M., Princeton University, Princeton, New Jersey.
CHEYNEY, DR. RALPH H., Biology Department, Brooklyn College, Brooklyn 10, New York.
CHILD, PROF. C. M., Jordan Hall, Stanford University, California.
CHURNEY, DR. LEON, Dept. of Physiology, Louisiana State University School of Medicine, New Orleans 13, Louisiana.
CLAFF, MR. C. LLOYD, 31 West Street, Randolph, Massachusetts.
CLARK, PROF. E. R., Wistar Institute, Woodland Avenue and 36th Street, Philadelphia 4, Pennsylvania.
CLARK, DR. LEONARD B., Department of Biology, Union College, Schenectady, New York.
CLARKE, DR. G. L., Department of Biology, Harvard University, Cambridge 38, Mass.
CLELAND, PROF. RALPH E., Indiana University, Bloomington, Indiana.
CLOWES, DR. G. H. A., Eli Lilly and Company, Indianapolis, Indiana.
COE, PROF. W. R., Yale University, New Haven, Connecticut.
COHN, DR. EDWIN J., 183 Brattle Street, Cambridge, Massachusetts.
COLE, DR. ELBERT C., Department of Biology, Williams College, Williamstown, Massachusetts.
COLE, DR. KENNETH S., University of Chicago, Chicago, Illinois.
COLLETT, DR. MARY E., Western Reserve University, Cleveland, Ohio.
COLTON, PROF. H. S., Box 601, Flagstaff, Arizona.
COLWIN, DR. ARTHUR L., Queens College, Flushing, Long Island, New York.
COOPER, DR. KENNETH W., Department of Biology, Princeton University, Princeton, New Jersey.
COPELAND, DR. D. E., Department of Zoology, Brown University, Providence, Rhode Island.
COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.
COSTELLO, DR. DONALD P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.

COSTELLO, DR. HELEN MILLER, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.

CRAMPTON, PROF. H. E., American Museum of Natural History, New York City, New York.

CRANE, JOHN O., Woods Hole, Massachusetts.

CRANE, MRS. W. MURRAY, Woods Hole, Massachusetts.

CROASDALE, HANNAH T., Dartmouth College, Hanover, New Hampshire.

CROUSE, DR. HELEN V., University of Pennsylvania, Philadelphia, Pennsylvania.

CROWELL, DR. P. S., JR., Department of Zoology, Miami University, Oxford, Ohio.

CURTIS, DR. MAYNIE R., 377 Dexter Trail, Mason, Michigan.

CURTIS, PROF. W. C., University of Missouri, Columbia, Missouri.

DAN, DR. KATSUMA, Misaki Biological Station, Misaki, Japan.

DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Virginia.

DAWSON, DR. A. B., Harvard University, Cambridge, Massachusetts.

DAWSON, DR. J. A., The College of the City of New York, New York City, New York.

DEDERER, DR. PAULINE H., Connecticut College, New London, Connecticut.

DEMEREC, DR. M., Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York.

DILLER, DR. WILLIAM F., 1016 South 45th Street, Philadelphia, Pennsylvania.

DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, West Virginia.

DOLLEY, PROF. WILLIAM L., University of Buffalo, Buffalo, New York.

DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

DOTY, DR. MAXWELL S., Northwestern University, Department of Botany, Evanston, Illinois.

DUBOIS, DR. EUGENE F., Cornell University Medical College, New York City, New York.

DUGGAR, DR. BENJAMIN M., c/o Lederle Laboratories Inc., Pearl River, New York.

DUNGAY, DR. NEIL S., Carleton College, Northfield, Minnesota.

DURYEE, DR. WILLIAM R., Dept. of Terrestrial Magnetism, Washington 15, D. C.

ELLIS, DR. F. W., 1175 Centre Street, Newton, Massachusetts.

EVANS, DR. TITUS C., Radiation Research Laboratory, College of Medicine, Iowa City, Iowa.

FAILLA, DR. G., College of Physicians and Surgeons, New York City, New York.

FAURÉ-FREMIET, PROF. EMMANUEL, Collège de France, Paris, France.

FAUST, DR. ERNEST C., Tulane University of Louisiana, New Orleans, Louisiana.

FERGUSON, DR. JAMES K. W., Department of Pharmacology, University of Toronto, Ontario, Canada.

FIGGE, DR. F. H. J., Lombard and Greene Streets, Baltimore, Maryland.

FISCHER, DR. ERNST, Baruch Centre of Physical Medicine, Medical College of Virginia, Richmond 19, Virginia.

FISHER, DR. JEANNE M., Department of Biochemistry, University of Toronto, Toronto, Canada.

FISHER, DR. KENNETH C., Department of Biology, University of Toronto, Toronto, Canada.
FORBES, DR. ALEXANDER, Harvard University Medical School, Boston, Massachusetts.
FRISCH, DR. JOHN A., Canisius College, Buffalo, New York.
FURTH, DR. JACOB, V. A. Hospital (Lisbon) Laboratories, Dallas, Texas.
GALTSOFF, DR. PAUL S., 420 Cumberland Avenue, Somerset, Chevy Chase, Maryland:
GARREY, PROF. W. E., Vanderbilt University Medical School, Nashville, Tennessee.
GASSER, DR. HERBERT, Director, Rockefeller Institute, New York City, New York.
GATES, DR. REGINALD R., Woods Hole, Massachusetts.
GEISER, DR. S. W., Southern Methodist University, Dallas, Texas.
GERARD, PROF. R. W., The University of Chicago, Chicago, Illinois.
GLASER, PROF. O. C., Amherst College, Amherst, Massachusetts.
GOLDFORB, PROF. A. J., College of the City of New York, New York City, New York.
GOODCHILD, DR. CHAUNCEY G., State Teachers College, Springfield, Missouri.
GOODRICH, PROF. H. B., Wesleyan University, Middletown, Connecticut.
GOTTSCHALL, DR. GERTRUDE Y., 315 East 68th Street, New York 21, New York.
GOULD, DR. H. N., Newcomb College, New Orleans 18, Louisiana.
GRAHAM, DR. J. Y., Roberts, Wisconsin.
GRAND, CONSTANTINE G., Biology Department, Washington Square College, New York University, Washington Square, New York City, New York.
GRANT, DR. MADELEINE P., Woods Hole, Massachusetts.
GRAVE, PROF. B. H., DePauw University, Greencastle, Indiana.
GRAY, PROF. IRVING E., Duke University, Durham, North Carolina.
GREGG, DR. J. R., Department of Zoology, Columbia University, New York 27, New York.
GREGORY, DR. LOUISE H., Barnard College, Columbia University, New York City, New York.
GRUNDFEST, DR. HARRY, Columbia University College of Physicians and Surgeons, New York City, New York.
GUDERNATSCH, DR. J. FREDERICK, New York University, 100 Washington Square, New York City, New York.
GUTHRIE, DR. MARY J., University of Missouri, Columbia, Missouri.
GUYER, PROF. M. F., University of Wisconsin, Madison, Wisconsin.
HAGUE, DR. FLORENCE, Sweet Briar College, Sweet Briar, Virginia.
HALL, PROF. FRANK G., Duke University, Durham, North Carolina.
HAMBURGER, DR. VIKTOR, Department of Zoology, Washington University, St. Louis, Missouri.
HAMILTON, DR. HOWARD L., Iowa State College, Ames, Iowa.
HANCE, DR. ROBERT T., The Cincinnati Milling Machine Co., Cincinnati 9, Ohio.
HARMAN, DR. MARY T., Kansas State Agricultural College, Manhattan, Kansas.
HARNLY, DR. MORRIS H., Washington Square College, New York University, New York City, New York.
HARRISON, PROF. ROSS G., Yale University, New Haven, Connecticut.
HARTLINE, DR. H. KEFFER, University of Pennsylvania, Philadelphia, Pennsylvania.

HARTMAN, DR. FRANK A., Hamilton Hall, Ohio State University, Columbus, Ohio.
HARVEY, DR. E. NEWTON, Guyot Hall, Princeton University, Princeton, New Jersey.
HARVEY, DR. ETHEL BROWNE, 48 Cleveland Lane, Princeton, New Jersey.
HAUSCHKA, DR. T. S., Institute for Cancer Research, Philadelphia 30, Pennsylvania.
HAYDEN, DR. MARGARET A., Wellesley College, Wellesley, Massachusetts.
HAYES, DR. FREDERICK R., Zoological Laboratory, Dalhousie University, Halifax, Nova Scotia.
HAYWOOD, DR. CHARLOTTE, Mount Holyoke College, South Hadley, Massachusetts.
HECHT, DR. SELIG, Columbia University, New York City, New York.
HEILBRUNN, DR. L. V., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.
HENSHAW, DR. PAUL S., National Cancer Institute, Bethesda, Maryland.
HESS, PROF. WALTER N., Hamilton College, Clinton, New York.
HIBBARD, DR. HOPE, Department of Zoology, Oberlin College, Oberlin, Ohio.
HILL, DR. SAMUEL E., 18 Collins Avenue, Troy, New York.
HINRICHS, DR. MARIE, Department of Physiology and Health Education, Southern Illinois Normal University, Carbondale, Illinois.
HISAW, DR. F. L., Harvard University, Cambridge, Massachusetts.
HOADLEY, DR. LEIGH, Biological Laboratories, Harvard University, Cambridge, Massachusetts.
HÖBER, DR. RUDOLF, University of Pennsylvania, Philadelphia, Pennsylvania.
HODGE, DR. CHARLES, IV, Temple University, Department of Zoology, Philadelphia, Pennsylvania.
HOGUE, DR. MARY J., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
HOLLAENDER, DR. ALEXANDER, P.O. Box W., Clinton Laboratories, Oak Ridge, Tennessee.
HOPKINS, DR. DWIGHT L., Mundelein College, Chicago, Illinois.
HOPKINS, DR. HOYT S., New York University, College of Dentistry, New York City, New York.
HYMAN, DR. LIBBIE H., American Museum of Natural History, New York City, New York.
IRVING, LT. COL. LAURENCE, Swarthmore College, Department of Zoology, Swarthmore, Pennsylvania.
ISELIN, MR. COLUMBUS O'D., Woods Hole, Massachusetts.
JACOBS, PROF. MERKEL H., School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
JENKINS, DR. GEORGE B., 1336 Parkwood Place, N.W., Washington, D. C.
JOHLIN, DR. J. M., Vanderbilt University Medical School, Nashville, Tennessee.
JONES, DR. E. RUFFIN, University of Florida, Gainesville, Florida.
KAAN, DR. HELEN W., National Research Council, 2101 Constitution Avenue, Washington, D. C.
KAUFMANN, PROF. B. P., Carnegie Institution, Cold Spring Harbor, Long Island, New York.
KEMPTON, PROF. RUDOLF T., Vassar College, Poughkeepsie, New York.

KIDDER, DR. GEORGE W., Amherst College, Amherst, Massachusetts.
KIDDER, JEROME F., Woods Hole, Massachusetts.
KILLE, DR. FRANK R., Carleton College, Northfield, Minnesota.
KINDRED, DR. J. E., University of Virginia, Charlottesville, Virginia.
KING, DR. HELEN D., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.
KING, DR. ROBERT L., State University of Iowa, Iowa City, Iowa.
KLOTZ, DR. I. M., Department of Chemistry, Northwestern University, Evanston, Illinois.
KNOWLTON, PROF. F. P., Syracuse University, Syracuse, New York.
KOPAC, DR. M. J., Washington Square College, New York University, New York City, New York.
KRAHL, DR. M. E., Washington University School of Medicine, Department of Pharmacology, St. Louis, Missouri.
KRIEG, DR. WENDELL J. S., 303 East Chicago Ave., Chicago, Illinois.
LANCEFIELD, DR. D. E., Queens College, Flushing, New York.
LANCEFIELD, DR. REBECCA C., Rockefeller Institute, New York City, New York.
LANDIS, DR. E. M., Harvard Medical School, Boston, Massachusetts.
LANGE, DR. MATHILDE M., Wheaton College, Norton, Massachusetts.
LAVIN, DR. GEORGE I., Rockefeller Institute, New York City, New York.
LAZAROW, DR. ARNOLD, Western Reserve University School of Medicine, Cleveland 6, Ohio.
LEWIS, PROF. I. F., University of Virginia, Charlottesville, Virginia.
*LILLIE, PROF. FRANK R., The University of Chicago, Chicago, Illinois.
LILLIE, PROF. RALPH S., The University of Chicago, Chicago, Illinois.
LITTLE, DR. E. P., Phillips Exeter Academy, Exeter, New Hampshire.
LOCHHEAD, DR. JOHN H., Department of Zoology, University of Vermont, Burlington, Vermont.
LOEB, PROF. LEO, 40 Crestwood Drive, St. Louis, Missouri.
LOEB, DR. R. F., Department of Medicine, College of Physicians and Surgeons, New York City, New York.
LOEWI, PROF. OTTO, 155 East 93d Street, New York City, New York.
LOWTHER, MRS. FLORENCE DEL., Barnard College, Columbia University, New York City, New York.
LUCAS, DR. ALFRED M., Regional Poultry Research Laboratory, East Lansing, Michigan.
LUCKÉ, PROF. BALDUIN, University of Pennsylvania, Philadelphia, Pennsylvania.
LYNCH, DR. CLARA J., Rockefeller Institute, New York City, New York.
LYNCH, DR. RUTH STOCKING, Dept. of Zoology, University of California, Los Angeles 24, California.
LYNN, DR. WILLIAM G., Department of Biology, The Catholic University of America, Washington, D. C.
MACDOUGALL, DR. MARY S., Agnes Scott College, Decatur, Georgia.
MACNAUGHT, MR. FRANK M., Marine Biological Laboratory, Woods Hole, Massachusetts.
MCCOUCH, DR. MARGARET SUMWALT, University of Pennsylvania Medical School, Philadelphia, Pa.

* Deceased.

McGregor, Dr. J. H., Columbia University, New York City, New York.
Macklin, Dr. Charles C., School of Medicine, University of Western Ontario, London, Canada.
Magruder, Dr. Samuel R., Department of Anatomy, Tufts Medical School, Boston, Massachusetts.
Malone, Prof. E. F., 153 Cortland Avenue, Winter Park, Florida.
Manwell, Dr. Reginald D., Syracuse University, Syracuse, New York.
Marsland, Dr. Douglas A., Washington Square College, New York University, New York City, New York.
Martin, Prof. E. A., Department of Biology, Brooklyn College, Brooklyn, New York.
Mathews, Prof. A. P., Woods Hole, Massachusetts.
Matthews, Dr. Samuel A., Thompson Biological Laboratory, Williams College, Williamstown, Massachusetts.
Mavor, Prof. James W., 24 Edward Street, Belmont, Massachusetts.
Mazia, Dr. Daniel, University of Missouri, Department of Zoology, Columbia, Missouri.
Medes, Dr. Grace, Lankenau Research Institute, Philadelphia, Pennsylvania.
Meigs, Mrs. E. B., 1736 M Street, N.W., Washington, D. C.
Memhard, Mr. A. R., Riverside, Connecticut.
Menkin, Dr. Valy, Department of Surgical Research, Temple University Medical School, Philadelphia, Pennsylvania.
Metz, Dr. C. B., Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.
Metz, Prof. Charles W., University of Pennsylvania, Philadelphia, Pennsylvania.
Michaelis, Dr. Leonor, Rockefeller Institute, New York City, New York.
Miller, Dr. J. A., Emory University, Atlanta 3, Georgia.
Milne, Dr. Lorus J., Zoology Department, University of Vermont, Burlington, Vermont.
Minnich, Prof. D. F., Department of Zoology, University of Minnesota, Minneapolis, Minnesota.
Mitchell, Dr. Philip H., Brown University, Providence, Rhode Island.
Moore, Dr. Carl R., The University of Chicago, Chicago, Illinois.
Moore, Dr. J. A., Barnard College, New York City, New York.
Morgan, Dr. Isabel M., Poliomyelitis Research Center, Baltimore 5, Maryland.
Morrill, Prof. C. V., Cornell University Medical College, New York City, New York.
Muller, Prof. H. J., Department of Zoology, Indiana University, Bloomington, Indiana.
Nabrit, Dr. S. M., Atlanta University, Morehouse College, Atlanta, Georgia.
Nachmansohn, Dr. D., College of Physicians and Surgeons, New York City, New York.
Navez, Dr. Albert E., Department of Biology, Milton Academy, Milton, Massachusetts.
Newman, Prof. H. H., 173 Devon Drive, Clearwater, Florida.
Nichols, Dr. M. Louise, Rosemont, Pennsylvania.

*Nonidez, Dr. José F., Cornell University Medical College, New York City, New York.

Northrop, Dr. John H., The Rockefeller Institute, Princeton, New Jersey.

Ochoa, Dr. Severo, New York University, College of Medicine, New York 16, New York.

Oppenheimer, Dr. Jane M., Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania.

Osburn, Prof. R. C., Ohio State University, Columbus, Ohio.

Oster, Dr. Robert H., University of Maryland, School of Medicine, Baltimore, Maryland.

Osterhout, Prof. W. J. V., Rockefeller Institute, New York City, New York.

Osterhout, Mrs. Marian Irwin, Rockefeller Institute, New York City, New York.

Packard, Dr. Charles, Marine Biological Laboratory, Woods Hole, Massachusetts.

Page, Dr. Irvine H., Cleveland Clinic, Cleveland, Ohio.

Pappenheimer, Dr. A. M., 5 Acacia Street, Cambridge, Massachusetts.

Parker, Prof. G. H., Harvard University, Cambridge, Massachusetts.

Parmenter, Dr. C. L., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Parpart, Dr. Arthur K., Princeton University, Princeton, New Jersey.

Patten, Dr. Bradley M., University of Michigan Medical School, Ann Arbor, Michigan.

Payne, Prof. F., University of Indiana, Bloomington, Indiana.

Peebles, Prof. Florence, 380 Rosemont Avenue, Pasadena, California.

Pierce, Dr. Madelene E., Vassar College, Poughkeepsie, New York.

Pinney, Dr. Mary E., Milwaukee-Downer College, Milwaukee, Wisconsin.

Plough, Prof. Harold H., Amherst College, Amherst, Massachusetts.

Pollister, Dr. A. W., Columbia University, New York City, New York.

Pond, Dr. Samuel E., 53 Alexander Street, Manchester, Connecticut.

Pratt, Dr. Frederick H., Wellesley Hills 82, Massachusetts.

Prosser, Dr. C. Ladd, University of Illinois, Urbana, Illinois.

Ramsey, Dr. Robert W., University of Virginia Medical School, Richmond, Virginia.

Rand, Dr. Herbert W., Harvard University, Cambridge, Massachusetts.

Rankin, Dr. John S., Zoology Department, University of Connecticut, Storrs, Connecticut.

Redfield, Dr. Alfred C., Harvard University, Cambridge, Massachusetts.

Reid, Dr. W. M., Monmouth College, Monmouth, Illinois.

Renn, Dr. Charles E., Sanitary Laboratories, The Johns Hopkins University, Baltimore, Maryland.

Renshaw, Dr. Birdsey, Department of Physiology, University of Oregon Medical School, Portland, Oregon.

Reznikoff, Dr. Paul, Cornell University Medical College, New York City, New York.

Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, Ohio.

* Deceased.

RICHARDS, PROF. A., University of Oklahoma, Norman, Oklahoma.
RICHARDS, DR. A. GLENN, Entomology Department, University Farm, Univ. of Minnesota, St. Paul 8, Minnesota.
RICHARDS, DR. O. W., Research Department, American Optical Co., Buffalo, New York.
RIGGS, LAWRASON, JR., 120 Broadway, New York City, New York.
ROBBIE, DR. W. A., University Hospital, Iowa City, Iowa.
ROGERS, PROF. CHARLES G., Oberlin College, Oberlin, Ohio.
ROGICK, DR. MARY D., College of New Rochelle, New Rochelle, New York.
ROMER, DR. ALFRED S., Harvard University, Cambridge, Massachusetts.
ROOT, DR. R. W., Department of Biology, College of the City of New York, New York City, New York.
ROOT, DR. W. S., College of Physicians and Surgeons, Department of Physiology, New York City, New York.
RUEBUSH, DR. T. K., Dayton, Virginia.
RUGH, DR. ROBERTS, Department of Biology, Washington Square College, New York University, New York City, New York.
RYAN, DR. FRANCIS J., Columbia University, New York City, N. Y.
SAMPSON, DR. MYRA M., Smith College, Northampton, Massachusetts.
SASLOW, DR. GEORGE, Washington University Medical School, St. Louis, Missouri
SAUNDERS, LAWRENCE, W. B. Saunders Publishing Company, Philadelphia, Pennsylvania.
SCHAEFFER, DR. ASA A., Biology Department, Temple University, Philadelphia, Pennsylvania.
SCHARRER, DR. ERNST A., Department of Anatomy, University of Colorado School of Medicine and Hospitals, Denver, Colorado.
SCHECHTER, DR. VICTOR, College of the City of New York, New York City, New York.
SCHMIDT, DR. L. H., Christ Hospital, Cincinnati, Ohio.
SCHMITT, PROF. F. O., Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.
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WEISS, DR. PAUL A., Department of Zoology, The University of Chicago, Chicago, Illinois.
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WILLIER, DR. B. H., Department of Biology, Johns Hopkins University, Baltimore, Maryland.
WILSON, DR. J. W., Brown University, Providence, Rhode Island.
WITSCHI, PROF. EMIL, Department of Zoology, State University of Iowa, Iowa City, Iowa.
WOLF, DR. ERNST, Biological Laboratories, Harvard University, Cambridge, Massachusetts.
WOODWARD, DR. ALVALYN E., Zoology Department, University of Michigan, Ann Arbor, Michigan.
WRINCH, DR. DOROTHY, Smith College, Northampton, Massachusetts.
YNTEMA, DR. C. L., Department of Anatomy, Syracuse University Medical College, Syracuse, New York.
YOUNG, DR. B. P., Cornell University, Ithaca, New York.
YOUNG, DR. D. B., 7128 Hampden Lane, Bethesda, Maryland.
ZINN, DR. DONALD J., Rhode Island State College, Kingston, Rhode Island.

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PROTOPLASMIC VISCOSITY CHANGES DURING MITOSIS IN THE EGG OF CHAETOPTERUS

L. V. HEILBRUNN AND W. L. WILSON

Department of Zoology, University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole, Massachusetts [1]

In spite of the enormous effort that has been spent in order to discover the basic cause of cancer, a disease primarily due to the initiation of cell division in cells which normally do not divide, there has been but little advance in the past twenty years in our understanding of the basic physiology of cell division. Of the three main theories which have been proposed to account for the initiation of cell division, only one survives and that has had but little test. The idea that cell division is caused by an increase in cell permeability can scarcely be held at the present time, for in marine egg cells the calcium ion is a potent agent for promoting mitosis (See Pasteels, 1941; Hollingsworth, 1941) and the calcium ion is well known for its effect in decreasing rather than increasing cell permeability. Secondly, at the present time it can hardly be maintained that an increase in respiration is the primary cause of initiation of cell division. When certain types of cells are incited to divide, the respiration does increase, but other types of cells show no such effect, and in still other cells the respiration decreases (Whitaker, 1931a, b, c; 1933a, b; compare also Holter and Zeuthen, 1944). Nor, on the basis of present evidence, can it be held that a particular respiratory system is involved; at any rate the attempt to argue that in the sea-urchin egg the cytochrome oxidase system is activated when the cell is incited to divide is rather an expression of wishful thinking than of careful experimentation (See Robbie, 1946). We are left then with the third of the three major theories, the view that the primary impetus to cell division is a mitotic gelation akin to the gelation which occurs generally in cells when they respond to stimulation. This colloidal theory of cell division is discussed at some length in the second edition of Heilbrunn's Outline of General Physiology (1943, see Chapt. 42).

One of the reasons for believing in the colloidal theory is that in those cells in which viscosity studies have been made, the appearance of the mitotic spindle appears to be preceded by a very definite gelation of the cytoplasm. Actually, however, only a few types of cells have been studied, and if this point is to be firmly established, we should have additional information for other types of cells.

We were led to a study of the egg of the annelid Chaetopterus pergamentaceus Cuvier, because this worm is found in suitable numbers at Woods Hole and can be obtained for study, and also because the egg represents a type similar to Cumingia in that fertilization occurs at the time of the first maturation spindle. Unfortunately, at the present time Cumingia is very rare at Woods Hole and therefore can not be used for experimental work.

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Chaetopterus eggs can be obtained throughout the summer (Compare Mead, 1898). One female worm provides enough eggs for several experiments. When the eggs are shed into the sea water, they are in the germinal vesicle stage, but as soon as they enter the sea water, maturation begins and proceeds until the metaphase stage of the first maturation division is reached. Then, if the egg is fertilized, the maturation divisions continue and cleavage follows.

Obviously, the Chaetopterus egg is convenient for study. We plan to use it in various types of experimental work. We were interested therefore in knowing the normal cycle of viscosity change and how this cycle was related to the mitotic phenomena occurring between the time of fertilization and the first cleavage. Strangely enough, in spite of the great amount of cytological work on mitosis, there is no complete minute by minute time record of what can be seen in fixed and sectioned material during the progressive stages of mitosis in marine eggs.

Methods

The sexes of Chaetopterus can be determined from the color of the gametes contained in the transparent parapodia. The eggs are yellow and the sperm are white. A few posterior segments of a worm were cut off and placed in a small stender dish containing about 20 ml. of sea water. The parapodia of these excised segments were cut open and as a result the eggs or the sperm, as the case might be, exuded into the sea water. Eggs were filtered through cheesecloth to remove the excess jelly and extraneous tissue and then were washed by decantation. The eggs are usually so abundant that only a few segments of the worm provide enough eggs for a single experiment. A sperm suspension was prepared by removing the parapodia and segments from the stender dish and then adding to the original 20 ml. another 10 ml. of sea water. Two or three drops of this suspension were used to fertilize one batch of eggs. A worm does not die following the removal of several segments and indeed the same worm may be used several times.

In all of our work, the eggs of only one female were used in any given series of experiments. The eggs were kept in a water bath maintained at a constant temperature of 21° C. In some experiments, the temperature varied slightly, but ordinarily the variation was not greater than two or three tenths of a degree. As soon as the eggs began to show indications of cleavage, counts were made as rapidly as possible in order to determine with reasonable precision the exact time at which 50 per cent of the eggs had divided. In these counts, all eggs in which a cleavage plane had begun to travel across the egg were regarded as cleaved. Actually, the passage of the cleavage plane through the egg takes an appreciable time, perhaps a minute or more, so that the cleavage times we recorded are somewhat less than the times would be if we considered as cleavage time the time at which the egg is completely divided. In making rapid counts on living eggs, it would scarcely be possible to use as a criterion of cleavage the complete division of the egg. At a temperature of 21° C. we found the average cleavage time to be 56 minutes.

Protoplasmic viscosity tests were made with an Emerson hand centrifuge. The handle of the centrifuge was turned once every two seconds. This represents a speed of 85 revolutions per second. The radius of turn was 8 cm. The centrifugal force was calculated to be 2325 times gravity. A few preliminary tests of protoplasmic viscosity during mitosis showed that the viscosity changes in the

dividing Chaetopterus egg are not as pronounced as they are in the egg of Cumingia or in the egg of Arbacia. If tests are to be made at frequent intervals, observations must be made rapidly. This can introduce uncertainty. We were worried over the possibility that subjective impressions might creep in. Accordingly, we decided on the following procedure. For any given test, the centrifuge was turned by Mrs. Jean Wilson. As soon as the turning was completed, she passed the centrifuge tube to one of us as quickly as possible. The eggs were then removed from the tube to two microscope slides. Each of us had a microscope, and we observed the centrifuged eggs independently. In this way, we were able to make tests at one minute intervals and although the observations were necessarily very rapid, when we compared our results at the end of a series of tests, we found remarkably good agreement.

When a Chaetopterus egg is centrifuged, lighter (presumably fat) granules move to the centripetal pole, and heavier yolk granules move to the centrifugal pole. There is a cortical layer of granules which does not move at all. Details of the appearance of centrifuged eggs are given by Lillie (1906). When we observed the centrifuged eggs, we recorded everything that we could see. If the viscosity is relatively low, the yolk granules move more readily through the egg; the fat granules also move more readily. It is possible to observe a shift of the yolk granules before any movement of the fat granules can be noted. One can follow viscosity change either by considering the movement of heavy or light granules. In order to obtain definite quantitative values for viscosity, we chose as an endpoint the appearance of a definite accumulation of fat granules at the centripetal pole of the egg. The number of seconds required to give this accumulation was used in plotting a viscosity curve.

The eggs of Chaetopterus are not very transparent. We wanted to know exactly what was happening in the mitotic process during every minute between fertilization and cleavage. This necessitated preparation of sections. Eggs were fixed at one minute intervals either in Bouin's fluid or in Meves' fluid. We fixed four complete series of eggs, and all four series were imbedded in paraffin. Sections were made from only one series (Bouin), the others being kept in reserve. Sectioning and staining were done by Miss Drusilla Van Hoesen. Our sections are eight microns thick and they are stained with Heidenhain's hematoxylin.

Results

The viscosity changes in the protoplasm of the Chaetopterus egg during the period between fertilization and cleavage are shown in Figure 1. The viscosity figures represent relative values and they have no absolute basis other than that they represent the number of seconds of centrifugation necessary to arrive at the endpoint described in the previous section. In order to obtain the viscosity values, after some preliminary tests, we ran through 12 complete series, in each of which the eggs were centrifuged at minute intervals for a given length of time. The times of centrifuging in the various tests were 4, 5, 7, 9, 10, 11, 12, 13, 14, 15, 17, and 18 seconds.

Fertilization is followed by a drop in viscosity; then while the maturation divisions are proceeding, the viscosity remains constant. In one or two of our series, we did get some indication that during the course of the maturation divisions, there

might be minor fluctuations in viscosity, but the weight of evidence is against any change whatsoever. This may seem strange, for in the egg of the clam Cumingia one of us had noted marked changes in viscosity during the maturation divisions (Heilbrunn, 1921). The difference between the egg of Chaetopterus and that of Cumingia is, however, easy to understand. In the relatively small egg of Cumingia, the maturation spindles occupy a rather large fraction of the egg volume. Thus Morris (1917) states that the first polar spindle "is large, and lies near the center of the egg. It might, in fact, be mistaken for a cleavage spindle in the metaphase, if it were not for the form of the chromosomes." Similarly, Jordan (1910) shows the first maturation spindle of Cumingia as a large structure extending through most of the egg; the distance between the centers of the centrospheres of this spindle is approximately half the diameter of the egg. On the other hand, the

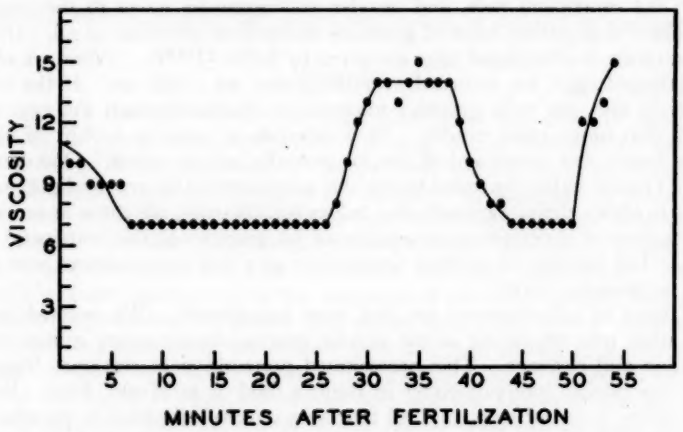

FIGURE 1. Protoplasmic viscosity changes in the egg of Chaetopterus during the time between fertilization and first cleavage.

maturation spindles of the Chaetopterus egg are relatively small. Thus, Lillie (1906) shows the fully formed maturation spindle of the Chaetopterus egg as small. In his Figures 3 and 4, the distance between the centers of its two centrospheres is only one-sixth of the diameter of the egg (Compare also Mead, 1898). Lillie (1906) showed that the cortex of the Chaetopterus egg was relatively viscous, containing granules which did not move when the egg was subjected to reasonably strong centrifugal force. We conceive, therefore, of the maturation spindles of the Chaetopterus egg as being relatively small bodies only several times as long as the cortical layer is thick and extending only a relatively short distance into the fluid region of the egg.

Following the maturation divisions, the protoplasm undergoes a sharp increase in viscosity. Our curve shows it to be approximately a two-fold increase. Some of our data indicated a somewhat greater change, but we preferred a conservative estimate. The viscosity increase is followed by a decrease in viscosity, and then

just before cleavage, the viscosity increases sharply again. These major changes in protoplasmic viscosity are related to the process of mitosis. In earlier work on the eggs of Cumingia and Arbacia, it was found that "the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity." It is of interest now to inquire as to whether the same correlations exist for the Chaetopterus egg.

Because of the fact that the Chaetopterus egg is one of the few invertebrate eggs that can conveniently be studied at Woods Hole, and because also of the present great interest in cell division, it was thought worth while to establish a complete time record of the mitotic changes in this egg as they occur during the interval between fertilization and first cleavage. One difficulty in presenting such a time record is the uncertainty of terminology. Mitosis is a continuous process and the various stages of this process can not be sharply delimited from each other. Moreover, not all authorities on mitosis agree in the way they define the stages. And even if a definition is rather uniformly followed, it is not always easy to apply it in such a way as to give a clear-cut decision as to when one stage ends and another begins. Thus, the telophase may be defined as the stage in which the chromosomes reach the poles of the spindle and begin to transform into vesicles or other structures characteristic of the resting stage. Now on a time basis, these two processes may not be simultaneous, and if one seeks to make sharp time distinctions, one must choose either the one or the other. Furthermore, the situation is complicated by the rapid succession of mitoses in an egg cell. Thus there may be no resting stage at all between two successive divisions, and there may not even be a complete telophase between the first and second maturation divisions.

For us it seemed wisest, if we were to present our results in tabular form, to make arbitrary criteria and distinctions. For our purposes, we shall define the metaphase as the stage in which the chromosomes are arranged along the equatorial plate of the spindle. We regard the anaphase as beginning as soon as the chromosomes have divided sufficiently so that we can see a space between the two groups of chromosomes. During anaphase, the two sets of chromosomes migrate toward the poles. It is hard to tell exactly when they have reached the poles. Accordingly, for this material, we chose as the distinction between anaphase and telophase, the moment when at least some of the chromosomes show signs of vesiculation. Actually, this distinction may depend to a slight extent on the depth to which the sections have been stained, but the difference between lightly and darkly stained sections does not appear to be great. On the basis of these distinctions, it was usually not too hard for us to tell when the egg cells were in metaphase, anaphase, or telophase. Prophases were more difficult. At the end of the first maturation division, after the first polar body has been separated off, there is an intermediate series of stages which are hard to classify. The polar body is given off 9 minutes after fertilization. At 10, 11, and 12 minutes after fertilization, one typically sees remnants of the first maturation spindle. At these times, the chromosomes are not vesiculated, so that according to our previous definition, it is not proper to call this stage a telophase. We might refer to it as an interphase, but we prefer to consider it as a late anaphase. At 13 minutes after fertilization, many half spindles appear in the sections. Following this is a stage in which the second maturation spindle appears; typically it lies in a plane perpendicular to the radius of the egg.

The chromosomes are frequently scattered along this second maturation spindle. This stage we refer to as the prophase of the second maturation spindle. The spindle then turns so that at metaphase it is perpendicular to the surface of the egg.

The second polar body is given off at 23 minutes after fertilization. The egg chromosomes now go into a very definite telophase stage and form discrete vacuoles. The egg nucleus is irregular in shape and may look like a bunch of grapes. At this stage, the male pronucleus, in sympathy as it were, may also become lobulated. There is thus a very definite telophase stage (at least in so far as the egg pronucleus is concerned). But between this telophase stage and the late prophase stage of the cleavage mitosis, it is hard to find distinctions which can be used for the purposes of our time scale. The male and the female pronuclei approach each other. There is thus a stage in which the pronuclei are separate and a stage when they are apposed. Usually, by the time they are apposed, the vesicular lobate appearance of the female pronucleus has disappeared so that this nucleus is now a smoothly spherical body with its chromatin either in a resting stage or in a condition indicative of an early prophase. There are exceptional cases in which the female pronucleus preserves its telophase appearance even though it is close to the male pronucleus. Then comes a stage in which the two pronuclei are fused together, or at least are apparently fused together. Mead (1898) says that an actual fusion of the pronuclei does not occur, but in many instances we were not able to detect a line of demarcation between the two pronuclei; and indeed in Mead's Figure 40, such a demarcation line is questionable. Accordingly, we distinguished a fusion nucleus stage. During this stage, the condition of the nucleus is almost certainly what most authors would call early prophase. There then comes a stage in which the nuclear membrane gradually breaks down and the chromosomes are arranged along the developing mitotic spindle; this stage is called late prophase.

In our time schedule, therefore, we distinguished the following stages: 1st maturation metaphase, 1st maturation anaphase, 2nd maturation prophase, 2nd maturation metaphase, 2nd maturation anaphase, 2nd maturation telophase, pronuclei separate, pronuclei apposed, fusion nucleus, late prophase of cleavage mitosis, metaphase, anaphase, and telophase (of cleavage mitosis). These stages are abbreviated in the headings of the table which gives a record of our findings. The data for the table were collected from a study of the slides prepared as described previously. For each minute of the time between fertilization and cleavage, we counted 20 cases in which the condition seemed clear in terms of one of the above-mentioned categories. The work was shared, and each of us made ten counts for each minute. On comparing our results, we found essential agreement.

The stages listed in our table do not give information on one point of considerable importance. They do not indicate at what moment the cleavage spindle first makes its appearance. After careful study of the sectioned material we have decided that the following series of events occurs. At 30 and 31 minutes after fertilization, the two pronuclei begin to come close to each other. There is at this time a large sperm aster with a large centrosphere. As the two pronuclei come still closer to each other (at 32 and 33 minutes after fertilization), between them they squeeze the centrosphere of the sperm aster into an elongated shape, so that it may form a narrow band between the two. This is the stage illustrated in Figure 39 of Mead (1898). This elongated centrosphere, with its astral rays

divided into two groups stretching well out into the cytoplasm, is not the definitive mitotic spindle, as subsequent stages indicate. Nevertheless, the line connecting the two sets of astral rays and the line along which the pronuclei fuse is almost always in the direction of the future spindle, for this line is typically perpendicular

TABLE I

Mitotic stages in the Chaetopterus egg as a function of time (minutes) at 21° C. Further explanation in text

Time	1st M	1st A	2nd P	2nd M	2nd A	2nd T	PNS	PNA	FN	LP	M	A	T
0	20												
1	20												
2	20												
3	20												
4	20												
5	20												
6	19	1											
7	12	8											
8	3	17											
9		20*											
10		20											
11		20											
12		19	1										
13		15	5										
14		8	8	4									
15			9	11									
16			3	17									
17				20									
18				19	1								
19				16	4								
20				13	7								
21				6	14								
22				1	19								
23					20*								
24					18	2							
25					8	12							
26						20							
27						18	2						
28						15	5						
29						14	4	2					

* Indicates time of appearance of 1st and 2nd polar bodies.

TABLE I—*Continued*

Time	1st M	1st A	2nd P	2nd M	2nd A	2nd T	PNS	PNA	FN	LP	M	A	T
30						8	9	3					
31						2	7	11					
32						1	5	14					
33							1	19					
34								20					
35								20					
36								9	11				
37								8	12				
38								3	17				
39								4	16				
40									13	7			
41									5	15			
42									4	15	1		
43										16	4		
44										15	5		
45										3	17		
46											19	1	
47											20		
48											18	2	
49											16	4	
50											3	17	
51												20	
52												20	
53												16	4
54												6	14
55												1	19
56													20

to the egg axis as indicated by the position of the polar bodies. This is also shown in Mead's Figure 39. Although at 32 and 33 minutes after fertilization, the astral rays are well developed, subsequently they seem to fade, so that 35 minutes after fertilization the asters either do not appear at all, or if present, they are faint. At this time there is no spindle. In the next three or four minutes, one occasionally sees instances of a double aster at one side of the fusion nucleus with what is apparently an embryonic spindle being stretched out between the two asters. Whether this is a general condition or not, only further study can decide. On one point we are certain, the definitive mitotic spindle does not appear until approximately 40 minutes after fertilization. In our study of eggs fixed at 40

minutes after fertilization, we found 15 out of 20 with the fusion nucleus elongated and pointed at its ends. In the pointed ends of these nuclei, spindle fibers show in 7 out of the 15 cases. Thus 7 out of 20 cells showed a true spindle. Probably this is somewhat less than the true proportion, for a nucleus might well be cut so that spindle fibers though present would not be visible. As far as our observations go, they indicate rather clearly that the moment at which the definitive mitotic spindle appears is 40 minutes after fertilization.

Let us now attempt to correlate our viscosity curve with the mitotic changes as shown by our observations of the fixed material. The viscosity curve shows a minimum of viscosity from 44 minutes after fertilization to 50 minutes after fertilization. This is almost exactly the time during which the cell is in metaphase, for the table shows the metaphase period to extend from 45 to 49 minutes after fertilization. We have chosen as the moment at which the definitive mitotic spindle appears as 40 minutes after fertilization. This is essentially simultaneous with the moment at which the viscosity of the protoplasm begins to drop.

We conclude therefore that the appearance of the cleavage spindle in the Chaetopterus egg is preceded by a period in which the protoplasm is relatively viscous. As soon as the spindle is formed, the viscosity drops. The metaphase is the stage at which the viscosity of the protoplasm is at a minimum.

Finally, we should like to express our admiration of the cytological study made by Mead in 1898. In general we confirm his findings. There are one or two minor points in which we might differ. In his Figure 46, which represents what we would call an anaphase, he shows some bodies in the equatorial plane of the spindle; these he calls nucleoli. Lillie (1906) in his Figure 25 illlustrates similar bodies which he labels as "chromatin masses cut off from the chromosomes." We have frequently seen these bodies in the equatorial plane of the spindle during the anaphase. However, our sections seem to indicate that they are neither nucleoli or chromosome fragments, but rather cytoplasmic granules which have pushed their way into the equatorial plane of the spindle. If this is correct, it is an observation which may have some importance in the interpretation of the mitotic spindle, but we made no careful study of the phenomenon. We should merely like to call the attention of the cytologists to it.

Discussion

Our results provide a suitable basis for further work on the protoplasm of the dividing Chaetopterus egg, and we hope in the future to study the action of radiation and other agents in terms of their effect on the protoplasmic viscosity.

The viscosity curve that we have plotted for the Chaetopterus egg is essentially the same as that reported earlier for Arbacia and Cumingia. Heilbrunn (1921) stated that "The viscosity changes in Arbacia and Cumingia are absolutely parallel. In each case the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity. And in both eggs division of the cell is immediately preceded by a viscosity increase." As a matter of fact, all authors who have made objective measurements of protoplasmic viscosity during mitosis are in substantial agreement. A survey of much of the literature is given by Carlson (1946).

If one excludes the work done by subjective and non-quantitative methods, there is only one discordant paper. Fry and Parks in 1934 published what we believe to be a masterpiece of distortion. They made a few centrifuge measurements and then used Heilbrunn's data in plotting their curves, stating that Heilbrunn's measurements were more complete and accurate than their own. After doing this, they insist in a final discussion that Heilbrunn is wrong. They reach this strange conclusion by misquoting and distorting the views not only of Heilbrunn but also of almost every other worker in the field. Actually, though Fry and Parks claimed to have copied Heilbrunn's curves, this is not exactly true. The rise in viscosity which Heilbrunn found to occur in the Arbacia egg ten minutes after fertilization (for a cleavage time of 50 minutes) is shifted by Fry and Parks so that it occurs approximately seven or eight minutes after fertilization (for a cleavage time of 67 minutes). Thus in Heilbrunn's curve, viscosity rises only after one-fifth of the time between fertilization and cleavage has elapsed, whereas in the curve stated by Fry and Parks to be a copy of Heilbrunn's curve, the rise occurs when about one-ninth of the time between cleavage and fertilization has elapsed. Needless to say, this shift favors the interpretation Fry and Parks endorse. Moreover, the final upsweep of the Arbacia curve is shifted so as to make the metaphase of division come in a period of high rather than low viscosity. Fry and Parks claim to find agreement between their curves, which they state to be Heilbrunn's curves, and Chambers' opinions on viscosity change during mitosis, opinions based on subjective microdissection studies of various species of eggs at uncertain times. This they do in order to make Heilbrunn's curves fit what Fry and Parks regard as Chambers' opinions. In their Chart 5, Fry and Parks credit Chambers with maintaining that the metaphase is a stage during which the protoplasm is fluid. But this is the exact opposite of what Chambers says. Thus Chambers (1919) states: "The time of appearance of the amphiaster until completion of cleavage lasts from 10 to 15 minutes. The increased viscosity of the egg during the amphiaster stage could be more easily demonstrated by the needle in the eggs of Echinarachnius and Cerebratulus than in those of Arbacia."

The facts of the case are as we have stated them, and no amount of distortion can hide the fact that the appearance of the mitotic spindle is preceded by a stage of high viscosity and followed by a stage of low viscosity. Heilbrunn (1921) suggested that "it is as though the spindle were coagulated out of the protoplasm." Recent work has indicated that in some types of proteins, gelation may result in the formation of a spindle-shaped structure called a tactoid (Bernal and Fankuchen, 1941). Bernal (1940) believes that the spindle is actually a tactoid.

Perhaps there are other correlations that may be made between changes in the protoplasm and the series of viscosity changes that we have described. For one thing, the stage of increasing viscosity occurs at a time when water is being taken from the cytoplasm by the enlarging pronuclei. Then, when the spindle appears, the nuclear membrane breaks down and this might involve an increase in the water content of the cytoplasm. Carlson (1946) suggests that changes in the viscosity of protoplasm during mitosis may be due to changes in the nucleic acid content of the cytoplasm. He thinks that a high content of nucleic acid in the cytoplasm would tend to produce a high viscosity. Carlson states that Brachet and also Painter found the cytoplasmic nucleic acids abundant in early prophase, less abundant or entirely absent from late prophase through anaphase, and increasing in

amount following division; but in the papers cited by Carlson it is not possible for us to find any data on the changes in nucleic acid content of the cytoplasm during various stages of mitosis. That there is an exact correlation between the amount of cytoplasmic nucleic acid and the protoplasmic viscosity is very doubtful, and certainly it has not in any sense been established. For one thing, the unfertilized sea-urchin egg is apparently rich in cytoplasmic nucleic acids (see, for example, Caspersson and Schultz, 1940), and yet this unfertilized egg has a low protoplasmic viscosity (Heilbrunn, 1920).

Summary

1. The viscosity of Chaetopterus egg protoplasm was determined at one minute intervals during the period between fertilization and cleavage.

2. By studying fixed, sectioned and stained material, the course of the mitotic processes in the Chaetopterus egg was followed minute by minute.

3. During the cleavage mitosis, marked changes in protoplasmic viscosity occur, and these are similar to the changes already described for the eggs of Arbacia and Cumingia.

4. The appearance of the mitotic spindle is preceded by an increase in protoplasmic viscosity and is followed by a decrease in protoplasmic viscosity.

5. During the metaphase, the protoplasmic viscosity is low.

6. Just before the cell divides, the protoplasmic viscosity increases markedly.

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INHIBITION OF FERTILIZATION IN ARBACIA BY BLOOD EXTRACTS

WILLIS E. PEQUEGNAT

Marine Biological Laboratory, Woods Hole, Massachusetts, and Department of Zoology, Pomona College, Claremont, California

From the time of publication of F. R. Lillie's paper (1914) on fertilization in Arbacia, some embryologists have maintained that the serum of Arbacia blood [1] provided an effective block to fertilization in this and a few other marine invertebrates. Lillie formulated the hypothesis that fertilization in Arbacia was actuated through the conjoining of certain constituents of egg and sperm by a substance called fertilizin, the presence of which in solution could be detected by the agglutinating action which it exerted upon sperm in aqueous suspension (see Tyler, 1948, for recent review of the subject). Asserting that filtered blood of Arbacia was capable of inhibiting fertilization while it did not prevent fertilizin from agglutinating sperm, Lillie linked this inhibitory action into his conception of the mechanism of fertilization by postulating that the serum-inhibitor prevented the uniting of fertilizin with the necessary constituent of the egg.

Oshima (1921) published the results of a few experiments which had motivated him to suggest that an external ("dermal") secretion was responsible for the inhibition observed by Lillie. That Oshima was not prepared to enter a complete denial of Lillie's conclusions is evidenced by his admitting that filtered blood was capable of exhibiting a weak though unpredictable inhibitory influence upon the fertilizability of the egg. Interestingly enough the degree of inhibitory action considered weak by Oshima fell within the range certainly considered significant by Lillie. Furthermore, it is worthy of note that Lillie was not able to offer a satisfactory explanation of the fact that the potency of undiluted blood samples displayed degrees of inhibitory effectiveness varying from zero to one hundred per cent. Nonetheless, largely through the influence of E. E. Just, little or no attention was paid to Oshima's suggestions by the majority of interested embryologists, except, perhaps, for Harvey (1939).

Apparently critical data confirming Lillie's conclusions were brought forth by Just (1922), who, at the same time, brushed aside Oshima's contraindications without any statement that he had attempted to repeat the latter's experiments. Also, Just stated that the most plausible explanation of Oshima's results would depend upon the presence of excretory or defecatory wastes in his solutions.

The matter rested at this point until the summer of 1946 when, at the suggestion of Dr. Albert Tyler, Richard L. Murtland, Albert H. Banner, and the present

[1] It has been convenient to use the word blood as a synonym of the term perivisceral fluid, even though strict interpretation may not warrant the practice. For present purposes the words serum and plasma are considered as literal equivalents when applied to Arbacia, since in this organism the clot is believed to be purely of cellular composition. Since Lillie and Just had previously used serum to denote the material obtained from whole blood by clotting, filtering, or centrifugation, I have followed this choice entirely for the virtue of consistency.

author [2] collaborated briefly in repeating a few of Lillie's experiments. Becoming interested in the mechanism of this inhibitory action, I carried on alone during the latter part of the summer of 1946 and returned to Woods Hole in the summer of 1947 to proceed with the same problem.

Although at the time I was unaware of Oshima's publication, I undertook to verify Lillie's observations before proceeding to a study of the *modus operandi* of the inhibitor. Following Lillie's techniques as closely as possible, I obtained results which corroborated his. Thus, I was convinced that his conclusion to the effect that Arbacia serum contained a factor capable of inhibiting fertilization was valid. But when, during the summer of 1947, I introduced techniques of collecting blood designed to yield uncontaminated samples, I obtained results which revealed that Lillie's original description of the source of the inhibitor must be modified. During the course of this study, it was found that serum samples removed by syringe so that they were uncontaminated with drainage from the exterior of the test did not possess inhibitory activity. Furthermore, it was found that sea water extracts from the tests of intact Arbacia were not capable of inhibiting fertilization. Thus, it became evident that some step in Lillie's technique, which I had followed previously in obtaining corroboratory results, was responsible for the appearance of the inhibitor in the serum samples. Chosen as the most likely cause was the fact that prior to opening the perivisceral cavity both Lillie and Just rinsed their urchins in tap water, presumably to kill any sperm present on the test which would otherwise fertilize samples of eggs. I had noticed that the application of tap water, even when followed by a sea water rinse within a few seconds, caused a yellow substance to appear in the excess water draining from the test of the as yet intact animal. A detailed study of this phenomenon revealed that this yellow exudate was capable of inhibiting fertilization. Additional experiments revealed that the immediate source of this inhibitor was to be found in certain granules or cells located in the tube feet and a few other organs. And, contrary to the findings of Lillie and Just, the ultimate source of the inhibitor was found to be some of the blood cells found in the perivisceral fluid. The present paper gives the details of these experiments.

Method

The sea-urchin *Arbacia punctulata* was the principal animal used in these experiments. Perivisceral fluid was removed from Arbacia by methods designed both to permit contamination from the outside and by methods devised to prevent such contamination. In addition, various techniques were devised which might supply information relative to the ultimate source of the inhibitor. Also, one significant step not used by previous workers was added to the routine handling of all samples. Having noted that the pH of sea-urchin blood was lower than that of sea water, and since this in itself may interfere with fertilization (Tyler and Scheer, 1937), it was decided that the pH of all samples should be adjusted to that of sea water. Moreover, in order to obviate any modification of results arising from undue concentrations of egg or sperm secretions, all samples containing gametes were discarded.

In most experiments one drop each of eggs and sperms were introduced into 2 cc. of fluid, be it sea water or extract, in Syracuse dishes. In each instance the

[2] Three members of the 1946 Embryology Class of the Marine Biological Laboratory.

eggs from a single female were used for each series of experiments. The average concentration of eggs in suspension was found by actual count to be between 2200 and 2500 eggs per drop. Fresh sperm suspensions were used for fertilizations and were made by diluting one drop of dry sperm with the equivalent of 99 drops of sea water. The various fluids were tested in serial two-fold dilutions of 2 cc. down to 64-fold. Determinations of the percentage of fertilization were made by actual counts under low magnification from three to five hours after insemination. The practice of first scanning the dish and then counting between four and five hundred eggs along two diameters was followed consistently.

Most of the experiments with differently prepared fluids were run simultaneously, as is indicated by similar dates in the tables.

Experiments

Series I. This experiment was carried out essentially as outlined by Lillie and Just, as follows: (1) Arbacia rinsed in tap water for a few seconds, shaken and rinsed in filtered sea water; (2) animals permitted to drain, cut made with scissors around peristome, and fluid drained into Syracuse dish; (3) clot permitted to form, checked for presence of gametes, then filtered; and finally (4) samples were centrifuged lightly and the pH adjusted to that of sea water. The fluid was then used undiluted, or diluted as described above. In each instance the fluid obtained in this way had a yellowish tinge.

This series of experiments, involving a total of 42 animals, was repeated seven times between July 8 and August 2, 1947. From the results tabulated in the left half of Table I it can be observed that this solution was effective in blocking fertilization when used undiluted. An average of approximately 3 per cent fertilizations was obtained in undiluted fluids as compared to nearly 100 per cent fertilizations of eggs in sea water controls. A summary of part of Lillie's work (1914), in which undiluted fluid collected from 50 Arbacia in the same manner and within the above dates was used, gives an average of 50 per cent fertilizations, as compared to 97 per cent in the sea water controls. The apartness of our results can be explained in part by the fact that his samples were used individually, while in my experiments fluid from all individuals was pooled before being tested. For example, Lillie's data show that the serum obtained by him from one individual contained no inhibitor, while the serum from another contained enough to inhibit all eggs tested. This would yield an average of 50 per cent inhibition. On the other hand, if these two samples had been pooled before being tested, it is possible that sufficient inhibitor would be present in the mixture to give complete inhibition. A comparison of the effects of diluting the serum show this conjecture to be valid. Thus, the percentage of fertilization increased when the fluid collected on August 2 was diluted, as follows:

Percentage serum (in sea water)	100	50	25	12.5	6.2
Percentage fertilization	0	16	68	99	100

In this experiment I obtained an average of 32 per cent inhibition with a 25 per cent solution of serum, and Lillie's data show that he obtained 30 per cent inhibition when using a 20 per cent solution of serum obtained in the same manner.

Series II. The tap water rinse was eliminated in this experiment; otherwise all procedures were the same as those outlined in Series I. Again, fluid from a total of 42 animals was tested on seven occasions between July 10 and August 3, 1947. The fluid in each case was clear, not yellow.

The results, as tabulated in the right half of Table I, offer a marked contrast to those of Series I. There is no significant difference between the percentages of fertilizations obtained from eggs inseminated in undiluted serum and those inseminated in sea water (both yielding approximately 99 per cent fertilizations). Hence it became apparent that the application of tap water was linked in some manner with the appearance of the inhibitor. Additional experiments were performed in order to determine whether it was being liberated into the serum from within the animal or from the outside.

TABLE I

Serum obtained by cutting peristome

With tap water rinse (Series I)					Without tap water rinse (Series II)				
July	pH	Adjusted pH	Percentage fertilizations		July	pH	Adjusted pH	Percentage fertilizations	
			Serum (100%)	Sea water				Serum (100%)	Sea water
8	7.5	8.0	0	100	10	7.6	8.0	100	100
10	7.4	8.0	0.5	100	12	7.6	7.9	99	99
12	7.6	8.0	0	99	14	7.7	8.0	100	100
14	7.6	8.0	0	100	21	7.5	7.9	100	100
26	7.2	8.0	10	100	26	7.5	8.0	95	100
27	7.5	8.0	5	99	Aug. 2	7.6	8.0	100	100
Aug. 2	7.5	8.0	0	100	3	7.0	8.0	100	100

Series III. In order to circumvent contamination of serum with external drainage, the fluid was withdrawn by means of a 10 cc. syringe equipped with a No. 22 gauge hypodermic needle. The needle was introduced into the perivisceral cavity through the peristome about 3 mm. from Aristotle's lantern. Care had to be exercised to keep the needle from penetrating the gonads and to prevent the plunger from crushing blood cells when fluid was expelled from the syringe. When the proper depth of penetration had been determined, the needle was ensheathed with rubber tubing long enough to stop it at the desired level. All animals used in this experiment were rinsed momentarily in tap water and then in sea water prior to withdrawing the fluid. Subsequently, the fluid was prepared and used exactly as described previously. The fluid in all cases was clear, not yellow.

Eight experiments were performed with serum collected in this manner between July 8 and August 3. The data from this series are tabulated in Table II. An average of 99.1 per cent fertilizations was obtained from eggs fertilized in all undiluted samples of this serum. This evidence, when coupled with the results of Series I, indicated that the inhibitor evoked by tap water drained into the perivisceral fluid from the outside when the latter was collected by cutting the peristome. Moreover, there seems little reason for doubting that these facts explain

the large range of variation in potency of inhibitor recorded both by Lillie and Just, because the amount of drainage in their samples would have varied inversely with the time elapsing between rinsing the animals and withdrawing the fluid, and directly with the time required to drain each animal.

Thus far the following facts have been ascertained: (1) that the inhibitor of fertilization is not found in the serum of the intact Arbacia; (2) that tap water causes the inhibitor to appear in samples of serum collected by the method of Lillie and Just (Series I); and (3) that the inhibitor so evoked comes from the outside of the animal.

It is important to note that when inhibition has been observed up to this point perivisceral fluid plus external drainage have been in solution together. Further experiments were performed to reveal whether this complex was necessary for

TABLE II

Serum withdrawn by syringe after tap water rinse

(SERIES III)

July	pH	Adjusted pH	Percentage of fertilizations	
			Serum (100%)	Sea water
8	7.5	7.9	100	100
10	7.6	8.0	100	100
12	7.6	7.9	98	99
14	7.7	8.0	99.5	100
17	7.9	7.9	100	100
26	7.5	7.9	95	100
Aug.				
2	7.5	8.0	100	100
3	7.0	8.0	100	100

inhibition, or whether the yellow drainage alone was sufficient to cause inhibition of fertilization. The fourth series of experiments was devoted to this problem.

Series IV. Several Arbacia were washed in filtered sea water to remove debris and wastes, then rinsed under the tap for fifteen seconds, and finally submerged briefly in filtered sea water to correct hypotonicity. After being shaken the animals were placed edgewise in glass funnels fitted with moistened filter paper, and the yellow drainage collected in centrifuge tubes. An equal number of control animals was subjected to the same treatment except that no tap water rinse was administered. Material from any animals that proceeded to defecate or shed gametes was discarded. The animals were left in the funnels a maximum of 20 minutes, or until draining ceased. The filtrate was centrifuged, although no visible separation occurred, and the pH adjusted to that of sea water. The clear, yellow fluid was then used as before.

This experiment was repeated seven times between July 10 and August 2, using a total of 68 animals. The results shown in Table III indicate that this material alone is sufficient to bring about inhibition of fertilization. In only two instances was fertilization obtained in this material when undiluted, and then only an average of 2 per cent of the eggs were activated. These data show also that

TABLE III

(SERIES IV)

Yellow fluid obtained from the test by draining after tap water rinse

July	pH	Adjusted pH	Percentage of fertilizations							
			Fluid dilutions (per cent)						Rinse control 100	Sea water control 100
			100	50	25	12	6	3		
10	7.6	8.1	1	—	—	—	—	—	97	99
12	7.8	8.0	0	—	—	—	—	—	100	100
14	7.9	8.0	0	—	—	—	—	—	100	100
17	7.8	8.1	0	0	0	0	17	100	96	97
19	7.6	7.9	0	0	0	0	0	—	95	98
*27	7.5	7.0	0	0	0	0	0	0		
27	7.5	8.0	3	2	3	3	3	99	100	100
27	7.5	9.0	21	29	10	10	21	84		
Aug. 2	7.6	8.0	0	0	0	0	1	5	100	100

* The experiment of July 27 was carried out at three pH's. The inhibitor's effectiveness is apparently reduced at pH 9. Note also an increase of effectiveness with moderate dilution; this occurred at other pH's as well.

these solutions effectively blocked fertilization in dilutions ranging from 50 to 3 per cent by volume. As a check some of this material was mixed in varying amounts with perivisceral fluid which alone had no effect on fertilization. As was anticipated, the previously impotent serum now became inhibitory to fertilization in proportion to the amount of yellow drainage added (Table IV). No appreciable difference in the inhibitor's activity could be detected between that diluted with sea water and that diluted in serum. Since the liquid draining from the control animals (those not rinsed in tap water) was found to be ineffective in blocking fertilization, it appears unlikely that this inhibition is due to the presence of soluble wastes, at least as proposed by Just in answer to Oshima's report.

TABLE IV

(SERIES IV)

Inhibitor mixed with impotent serum

Percentage of components in mixture		Percentage fertilizations	
Serum	Inhibitor	Mixture	Sea water
95	5	98	100
90	10	78	—
85	15	24	—
80	20	2	—
75	25	0	—
70	30	0	—
50	50	0	100

Series V. Much more potent solutions of inhibitor were obtained by (1) placing urchins directly into distilled water to depths not exceeding the greatest circumference of the shell and permitting them to remain 15 minutes, and (2) adjusting the osmotic value of the solution with sea water concentrated by evaporation. Control animals were placed in sea water to soak for the same period of time as the test animals. The osmotic values of these test solutions were checked by comparing the diameters of test eggs with those of the controls. No significant variations were observed. In addition one control was composed of equal volumes of distilled water and sea water concentrated to half its original volume by evaporation.

Although this experiment was run on several occasions, only one will be described in detail since all were essentially the same. On July 31 half a dozen Arbacia were placed in succession into 40 cc. of distilled water and permitted to remain approximately 5 minutes each. Six control animals were placed into the same volume of sea water in the same manner. The distilled water was immediately colored yellow, while the sea water remained clear and colorless throughout. After filtration, centrifugation, and adjustment of osmotic value, 62 cc. of yellow fluid were obtained. This obviously represents a much greater dilution per animal than in previous experiments. But despite this fact this solution prevented fertilization completely in serial dilutions down to 1 per cent. The sea water in which control animals had stood gave 100 per cent fertilizations, as did the other control solution.

Series VI. In order to narrow down the locus of origin of the inhibitor, five animals were cut into halves along the oral-aboral axis. All internal organs were removed and the inside of the tests scrubbed in sea water with a brush. Following this the sectioned tests were soaked for one hour in sea water, which was not discolored in the process. Then the tests were rinsed for a few seconds in tap water and sea water, and permitted to drain into a clean finger bowl. The drainage was yellow. The spines on the tests were still moving when the fluid was removed after one hour. The pH was adjusted from 7.6 to 8.0, and the material tested. The following results were obtained:

Percentage of extract	100	50	25	12	6	3
Percentage of fertilizations	3	6	3	3	92	100

When the same tests were again rinsed in tap water and sea water and permitted to drain, only 2 cc. of fluid were obtained. When used undiluted this second drainage gave 10 per cent aberrant cleavages. Controls gave 98 and 99 per cent fertilizations, respectively. These results provided additional evidence that some external structure was the source of the inhibitor.

Series VII. A study of individual tube feet under the microscope revealed a layer of closely packed, yellow granules or cells just beneath the outer epithelium. These granules maintained their integrity while immersed in sea water. But when the sea water was replaced with tap water, all traces of yellow material disappeared from within the feet. Simultaneously with this disappearance, the water around the feet was colored bright yellow. It is important perhaps to note that this material diffused through the outer epithelium and did not pass into the lumen of the foot. Tests run on this yellow material proved that it possessed the property

of inhibiting fertilization in a manner similar to that observed previously. One experiment will be described in greater detail.

Part of the tube feet from the oral hemispheres of four Arbacia were removed. This was done by letting the animal attach to a glass plate and then pulling the plate away. The tube feet were soaked in several rinses of sea water, covered momentarily with tap water and then placed into 4 cc. of clean sea water. The latter water was immediately colored yellow. After five minutes the supernatant fluid was decanted and its volume noted to be 5 cc. It was then filtered and the pH raised from 7.6 to 8.0. It is worthy of note that this extract was brighter yellow than that obtained by placing the whole animal in distilled water. The color of the extract deepened to a certain extent when the pH was elevated. Interestingly enough I found subsequently that it became colorless at pH 4 and below, and a darker yellow at pH 8 and above.

This tube-foot extract proved to be very effective at blocking fertilization. In concentrations running from 100 to 12 per cent no fertilizations occurred, and only 1 per cent of the eggs was fertilized in dishes containing as little as 3 per cent extract in sea water. This degree of effectiveness is made more remarkable by the fact that because of the dilution intrinsic to the method of extraction the extract represented only 20 per cent by volume of the inhibitor solution that exuded from the tube feet. Hence, the 12 per cent solution in the series of 2 cc. dilutions would actually contain a maximum of 0.025 cc. of inhibitor, or approximately 1.25×10^{-5} cc. per egg.

Because the color of this extract was not the same shade as that obtained when intact animals were used, I searched for other sources. It was found that spines gave forth a small amount of inhibitor, but only from their bases where epithelium was to be found. The bodies and tips of the spines, which in many instances had no fleshy covering, gave up a purplish substance which had no significant effect on fertilization. When this substance was mixed in small amounts with the extract from tube feet, however, the latter assumed the color of the extract from the intact animals.

Series VIII. Further work revealed that the inhibitor was carried by at least one type of amoebocyte found in the perivisceral fluid. Blood was removed by cutting the peristome, but attention is called to the fact that the animals were not rinsed in tap water. And, instead of filtering the blood as before, the plasma or serum was separated from the cells by light centrifugation and then decanted into clean flasks. At this time, an equal volume of sea water was added to the clot in the tube and the two mixed by shaking and rapid centrifugation. Whereas the plasma was colorless, the supernatant sea water solution was the same bright yellow as the extract from the tube feet. The pH of the two solutions offered an additional point of contrast. Whereas the pH of the plasma was 7, that of the yellow extract was 6.3, despite the fact that the sea water was pH 7.9 at the time of its addition to the clot. It is suggested that this depression of pH was caused by the liberation of the acid contents of the colorless amoebocytes. Before being tested, both solutions were brought up to pH 8.

A further contrast of properties of these two solutions was observed when they were tested: the percentage of fertilization in solutions of plasma equalled that of the controls, in this case 99 per cent; the yellow extract, however, permitted no

fertilizations when used undiluted. A two-fold dilution of the extract permitted only 10 per cent of the eggs to be fertilized. These results showed clearly that the inhibitor was carried by certain blood cells, and in such a manner that it did not normally pass from them into the plasma. Attempts to isolate the specific type or types of blood cells that carried the inhibitor were nullified by the fact that no practical method was devised for preventing the blood from clotting. The methods usually employed to prevent clotting of vertebrate blood were found to be of no value. Nonetheless, it was possible to observe microscopically that upon cytolysis the amoebocytes with yellow spherules (for classification of blood cells, see Kindred, 1926) gave up a yellow substance which upon addition of acid became decolorized as does a solution of the inhibitor. Two additional observations also serve to link the inhibitor obtained by methods described previously with that obtained directly from the blood cells.

It was possible to demonstrate that the potency of inhibitor extracts obtained from blood clots of animals that had been soaked previously in distilled water was less than that obtained from untreated animals. For example, blood was removed by syringe from six animals which had been used just previously for obtaining inhibitor by soaking in distilled water (after the method of Series V). The blood was then centrifuged, the plasma decanted and replaced by sea water, and the mixture shaken and centrifuged rapidly. These solutions were tested with the result that no fertilizations were permitted in the extract obtained by soaking in distilled water; 50 per cent fertilizations were obtained from the undiluted sea water extract of the clot; and 100 per cent fertilizations were obtained in the plasma and sea water controls.

The reciprocal of the above was also found to be true, viz., that animals from which all possible perivisceral fluid had been removed by syringe produced weaker solutions of inhibitor obtained by application of tap water (after the method of Series IV). I took six animals from which pervisceral fluid had just been removed by syringe, and rinsed them briefly in tap water and sea water, and then placed them into funnels from which the drainage was collected. This drainage permitted an average of 32 per cent fertilizations when used undiluted.

All of the observations made in this series of experiments lend some support to the opinion that the yellow granules observed in the tube feet may actually be yellow amoebocytes that are free to move between tube feet and the perivisceral cavity.

Series IX. The following experiment was devised to show whether or not the inhibitory effect of blood extracts upon eggs was reversible. One drop of eggs was placed into 2 cc. of undiluted inhibitor solution contained in each of six Syracuse dishes. After insemination the dishes were placed in running sea water on the water table. No fertilizations resulted in any of the dishes. At the end of two hours, the inhibitor solution was pipetted from one of the dishes and the eggs washed twice in fresh sea water and then reinseminated. Eggs in the remaining dishes were handled in the same manner 4, 8, 12, 14 and 24 hours after being introduced into the inhibitor solution. A series of six control dishes contained approximately the same number of eggs in 2 cc. of sea water; these were fertilized in series after the same intervals of time. The results are tabulated in Table V. Although there are some indications that some eggs were damaged by standing in the inhibitor solution, there is definite evidence that this blocking of fertilization is reversible.

TABLE V

(SERIES IX)

Fertilization of blocked eggs after washing

Exposure to inhibitor (hours)	Percentage fertilizations		
	Before washing	After washing	Controls
2	0	97	100
4	0	92	98
8	0	95	96
12	0	*90	94
14	0	†99	98
24	0	90	93

* About 30 per cent exhibited polyspermy.
† About 10 per cent aberrant cleavages.

Series X. Eggs that were observed to develop in various dilutions of inhibitor had indicated that post-fertilization developmental processes were not appreciably affected, but one experiment was run to test this point more effectively. Eggs were inseminated in sea water and transferred as quickly as possible into dishes containing undiluted inhibitor. Subsequent examinations revealed no significant differences between those samples of eggs that were placed in inhibitor and those that remained in the sea water. The zygotes were kept in the original inhibitor solution until they had reached the swimming stage. At this time they were transferred to sea water and carried on to the pluteus stage. Two observations were made during this time: (1) there were some indications that the rate of development was retarded slightly by the inhibitor, and (2) that the plutei developing from inhibitor-treated zygotes were smaller than the controls. These observations were not investigated further.

DISCUSSION

The results obtained from Series II and III of experiments refute the validity of the conclusion of Lillie and Just that the serum of Arbacia blood normally contains an inhibitor to fertilization. Although Series VIII showed that the inhibitor was carried by certain blood cells, the results of Series II indicated that the inhibitor did not leave the blood cells to enter the serum. In fact, the inhibitor was obtained from the cells in Series VIII only after vigorous shaking and rapid centrifugation. Since it has been shown that the inhibitor can be evoked by the application of tap water to the outside of the animal, it may be concluded that the inhibitor observed by them entered their samples from the outside of their urchins as a result of the application of tap water. Furthermore, the supposed variations in potency of the inhibitor reported by Lillie and Just were shown to be illusory by the results of Series I and III.

Certainly the property of inhibition is not of itself particularly interesting for no doubt a large number of substances could be used to inhibit fertilization in Arbacia, but most of them would very likely be inimical in one way or another to the gametes. Therefore, the fact that eggs appear to be fundamentally unharmed

by exposures to this natural inhibitor ranging from a few seconds to many hours serves to heighten one's interest. It has been shown that eggs which have remained blocked up to 24 hours in this inhibitor can be fertilized, provided they are washed thoroughly in sea water and reinseminated. Moreover, Just (1922) reported that he obtained development in blocked eggs (inseminated in inhibitor solution) without reinsemination, so long as the eggs were washed within two hours after fertilization.

Except for a slight depression of the rate of development, this inhibitor exerted no appreciable influence upon post-fertilization changes in the egg. Eggs that were fertilized at one instant and transferred immediately to inhibitor proceeded to develop into normal blastulae; yet when eggs were introduced into potent inhibitor and sperm added as quickly as possible blocking was complete. This latter observation supports the hypothesis that the inhibitor acts at the surface of the egg. Other evidence may be brought to bear on this point.

The inhibitor appeared to remove part of the egg's jelly layer, in proportion to concentration or to the duration of exposure. Inferred at first from the observation that eggs tended during the period of contact with inhibitor to aggregate more compactly than eggs in sea water, this conclusion was strengthened by the addition of dilute solutions of Janus Green B. Furthermore, the jelly layer of Chaetopterus eggs exhibited a marked affinity for the Arbacia inhibitor by staining a deep yellow during exposure, but the jelly layer was not removed by it. It is interesting to note also that this yellow cast was not removed by subsequent washings. Because the inhibitor obtained from Arbacia was observed to prevent fertilization of Chaetopterus eggs, it is unfortunate that no attempt was made to determine their fertilizability after washing. This might well have revealed whether the inhibitor itself is yellow or is only associated with the pigment in solution. While referring to associated species, it is appropriate to record that the Arbacia inhibitor does suppress fertilization in the sand-dollar, Echinarachnius parma. This fact was reported by all previous workers. In addition, Just (1923) stated that the blood of this sand-dollar blocked the fertilization of its eggs. It is possible, however, that this observation is subject to the same criticism herein advanced against his interpretation of the Arbacia inhibitor, because I observed that tap water evoked a similar response from Echinarachnius. Unfortunately, I could find no complete description of the method he used in obtaining this fluid.

Normal fertilization membranes were seldom observed on eggs that were fertilized in fresh sea water after prolonged exposures to the inhibitor. It is possible that this condition resulted from simple aging of the eggs. But in some instances no membranes could be observed even after the eggs began to cleave. That this was not tight membrane development is attested to by those extreme cases in which the blastomeres rounded up and were as easily separable as those of eggs treated with Ca-free sea water.

It was more difficult to observe definitive effects of the inhibitor on sperm. Little positive evidence as yet obtained rules out the possibility that the inhibitor acts directly upon the sperm. But this position could be rendered less tenable by several observations. In the first place, sperms appeared to be stimulated to greater activity when in the presence of inhibitor; and, secondly, they continued to move about inhibited eggs long after all evidence of motility of sperm had dis-

appeared in the controls. One point in this connection that might be of value in future work is the fact that the sperms which persisted in activity longest appeared to have lost their ability to attach to the egg. Thus, they moved about aimlessly among the eggs without attempting to penetrate. Finally, Just (1922) reported the actual penetration of sperms into the cortex and cytoplasm of blocked eggs. Presumably these were the sperms that were able to consummate fertilization when such blocked eggs were washed within the two-hour limit but not reinseminated.

Differences of opinion have arisen concerning the interaction, if any, between inhibitor and fertilizin. Lillie (1914) concluded that the effects of the inhibitor could be nullified by mixing it with fertilizin. One cannot question the data from which he drew this conclusion, but it appears to the present author that the method that he used to obtain neutralization of the inhibitor permits another interpretation of his data. In order to combine fertilizin with inhibitor, Lillie mixed serum and whole eggs in the ratio of two parts serum to one part eggs. Time intervening, the mixture was filtered and the filtrate tested for inhibitory activity. His data show that the untreated serum permitted only 0.5 per cent of the eggs tested to be fertilized, while the treated serum permitted 99.0 per cent of the eggs tested to be fertilized. Lillie concluded that the fertilizin had neutralized the inhibitor in the serum. But it is possible that little or no inhibitor was left in the filtrate. The basis for this interpretation is supplied by an experiment not previously described.

I mixed 4 cc. of inhibitor solution, obtained in the manner of Lillie, with 2 cc. of a suspension of eggs computed to contain approximately 0.75 cc. of sea water (Solution 1). Another 4 cc. of the same inhibitor were mixed with 0.75 cc. of sea water (Solution 2). A third solution was prepared by adding 2 cc. of strong egg-water (known to agglutinate sperm) with another 2 cc. of the inhibitor solution (Solution 3). After an interval of twenty minutes, all tubes were centrifuged lightly and 2 cc. samples were removed carefully from the top of each tube, and tested in the usual manner along with sea water controls. The following results were obtained: Solution 1 gave 75 per cent fertilization; Solutions 2 and 3 gave 0.0 per cent fertilizations; and 99 per cent of the eggs in the sea water controls were fertilized. These data suggested that the inhibitor combined in some manner with the eggs. Also, the fact that no fertilization occurred in the mixture of inhibitor and egg-water (Solution 3) supports the contention that the fertilizin does not neutralize the inhibitor, at least in the same sense of the word as used by Lillie.

The results of this experiment (particularly from Solution 1) provide a tentative explanation of the retarded activity of eggs that have stood in dilutions of inhibitor for some time after insemination. It is obvious that individual eggs in any sample are affected differentially by the inhibitor; otherwise there could be no explanation of the interesting fact that in dilutions of inhibitor some eggs are fertilized while others are not. Furthermore, one should recall that blocked eggs may be reversed to a state of fertilizability by soaking them in sea water. Therefore, it is possible that eggs which at first have the minimum of inhibitor necessary to prevent fertilization give this up slowly when the diffusion gradient has reversed, as the result of the greater affinity of other eggs for inhibitor. Just what conditions in or on the egg account for this differential reaction to inhibitor, I cannot say.

Although it appeared that fertilizin exerted no appreciable influence upon the

activity of the inhibitor, some evidence was obtained which indicated that a reverse influence did exist. On the one hand, as indicated in the introductory paragraph of this report, Lillie asserted that the inhibitor did not reduce the ability of fertilizin to agglutinate sperms. On the other hand, I found that concentrated solutions of inhibitor, when mixed in the ratio of 1 : 3 with egg-water capable of agglutinating sperm, would reduce the time required for reversal of agglutination. When these substances were mixed in equal parts, the egg-water lost its ability to bring about agglutination.

An interesting aspect of this problem is revealed by the close parallel between certain properties shared by the inhibitor and material extracted by Tyler (1940) from Arbacia eggs. Both of these materials are yellow; both appear to negate the sperm-agglutinating power of filtered egg suspension; both exhibit tendencies to cause clumping of eggs; and, under certain conditions, both reduce the fertilizability of eggs. The material extracted from eggs, however, produces a visible precipitation membrane on the egg's jelly layer; no membrane of this type has as yet been observed upon applying the blood inhibitor. Nonetheless, this parallelism between the properties of these two extracts is such that further study is indicated.

Summary

1. Whole perivisceral fluid (blood) of Arbacia contains a substance capable of inhibiting fertilization.

2. Contrary to the conclusions of previous investigators, this inhibitor is not normally present in the serum. Rather certain blood cells, particularly the amoebocytes with yellow spherules, are the ultimate source of the inhibitor.

3. The inhibitor believed by Lillie and Just to be found in the serum of Arbacia blood actually entered their samples as a contaminant from the outside of their animals.

4. The external application of tap water causes the inhibitor to appear in the drainage from the test. Under these conditions the inhibitor emanates from yellow bodies found in the hypodermis of the tube feet.

5. The supposedly variable potency of inhibitor reported by previous workers can be explained by the technique used in obtaining samples, and the methods used in testing its strength. In reality former workers were testing inhibitor in varying dilutions rather than testing the potency of a standard amount of inhibitor.

6. This inhibitor does react with the egg's jelly layer and can modify the fertilization membrane in proportion to concentration and duration of exposure. Eggs that are inhibited for short intervals of time (1–4 hours) can be fertilized and will develop normally (i.e. with membranes, etc.), provided they are rinsed thoroughly in fresh sea water.

7. Fertilizin is believed to have little influence on the activity of the inhibitor beyond a simple dilution effect. On the other hand, the sperm-agglutinating power of fertilizin-bearing solutions can be reduced or nullified by the addition of sufficient inhibitor.

8. It is suggested that this blood inhibitor may be related to an egg-agglutinin extracted from the egg itself.

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CHANGES IN DENSITY, WEIGHT, CHLORIDE, AND SWIMBLADDER GAS IN THE KILLIFISH, FUNDULUS HETEROCLITUS, IN FRESH WATER AND SEA WATER

VIRGINIA S. BLACK

Department of Biology, Dalhousie University, Halifax

Introduction

The field of osmotic regulation in aquatic animals has received much attention during the last fifty years. In fish this work has been largely directed toward a study of the euryhaline species such as the eel and salmon (Krogh, 1939). The theories currently accepted for the maintenance of water and salt balance by normal fish in sea water and fresh water were first thoroughly reviewed by Smith (1932), and have been well summarized by Krogh (1939), Baldwin (1940), and Scheer (1948).

The killifish, *Fundulus*, has been used by many investigators, probably because it is one of the few small euryhaline genera which are available in quantity and adapt readily to aquarium life. The most extensive work dealing with the effect of density changes on *Fundulus* and other fish was carried out by Sumner (1905). His experiments are based mainly on viability of groups of fish in various salinities and fresh water. He also measured weight changes and changes in chloride in the water and in the tissues of *Fundulus* resulting from removal from sea water to fresh water and *vice versa*.

The present investigation was designed to obtain serial quantitative measurements of rapid adjustments of a marine fish to fresh water, and so construct a more complete picture of a reaction whose qualitative aspects are already known.

The author is deeply indebted to Dr. F. R. Hayes for invaluable assistance in formulating the problem and also for information and helpful suggestions regarding the measurements of density of fishes.

Material and General Procedure

Live specimens of *Fundulus heteroclitus*, commonly known as killifish, mummichog, or salt water "minnow," were obtained from salt water flats northeast of Halifax, Nova Scotia. This species is normally found in the sea, in estuaries, and in brackish waters. In the laboratory the fish were kept in large glass aquaria (10 inches by 17.5 inches) having a depth of water of 6.5 inches. Stock sea water was obtained from the Northwest Arm, Halifax. The tap water was derived from the Halifax civic water supply. An analysis of the water made in 1940 (Leverin, 1942) from samples at the pumping station is given in Table I.

Experiments were not begun until the fish had been in the laboratory for at least two days. Fish which had been in the laboratory more than a week were not used to begin a series of experiments. The fish were not fed during the period of the experiment, but stock fish were fed every two days on Aylmer's canned beef

TABLE I

Analysis of Halifax water supply, July 1940 (Leverin, 1942)

	Parts per million		Parts per million
Color	30.0	Bicarbonate (HCO_3)	None
Alkalinity as $CaCO_3$	None	Sulphate (SO_4)	6.6
Residue on evaporation dried at 110° C.	30.0	Chloride (Cl)	2.5
Silica (SiO_2)	4.0	Nitrate (NO_3)	0.35
Iron (Fe)	0.05	Total hardness as $CaCO_3$	18.8
Calcium (Ca)	5.7	Calcium hardness	14.3
Magnesium (Mg)	1.1	Magnesium hardness	4.5

TABLE II

Density, weight, chloride and swimbladder gases of F. heteroclitus *in sea water*

Date 1947	Sex	Weight grams	Density (see text)	Chloride m.eq./kilo wet tissue	Swimbladder		
					CO_2 %	O_2 %	Vol. swimbladder / Wt. of fish
Series I							
June 2	♂	8.28		42	6.2	11.9	0.060
	♀	6.34			6.0	10.1	0.040
	♀	4.50		58	2.3	13.1	0.060
4	♀	6.98		55	3.1	10.5	0.063
	♂	7.87		58	1.3	14.2	0.051
7	♀	6.86		66	0	12.6	0.044
	♀	7.15		60	2.7	10.2	0.050
9	♀	3.40	1.017	50	1.2	15.5	0.048
10	♀	6.90	1.027	55	0.6	12.1	0.061
Average		*6.5*		*56*	*2.6*	*12.2*	*0.053*
Series II							
July 21	♀	2.09		50	1.2	15.1	0.033
	♀	1.51		50			
	♂	4.43			3.6	17.3	0.056
	♀	10.18			3.2	14.0	0.054
	♂	1.87			2.3	21.4	0.029
22	♀	1.47		50	3.0	20.0	0.029
23	♀	1.67		55	1.1	15.1	0.031
	♀	1.26	1.026	52	0.8	12.5	0.032
	♂, ♀	1.74		46			
24	♂	1.71		51	1.3	17.5	0.039
Aug. 1	♀	1.54		58	0.5	11.8	0.045
	♀	4.72	1.024	60	0.8	10.7	0.044
	♂	1.00		60	0.2	11.9	0.045
Average		*2.7*		*53*	*1.6*	*15.2*	*0.040*

prepared for babies. Both sexes were used. Most of the fish were sexually mature. The range in weight of the fish was one to twelve grams, but an attempt was made to select fish of approximately the same weight for each series.

In all aquaria the water was circulated and aerated by fine streams of air bubbles. The water was maintained at a pH between 7.0 and 8.5. The temperature varied from 15° C. to 19° C., but changes were taken into account in the density determinations.

Weight and density measurements were made on groups of two or three fish, taken as a unit, before the fish were transferred from sea water to the tap water (soft water) aquarium. The transition from sea water to fresh water, or the reverse, was always direct; no gradual acclimatization was undertaken. After a known number of hours or days the weight and density measurements were again made. The groups of fish were identified by the manner in which the tail fin was clipped.

Swimbladder gases and chloride content of the fish in the sea water aquarium were determined every other day during the course of a series of experiments and provided the data for "normal sea water fish" (Table II). Swimbladder gases and chloride content of the experimental fish in tap water were determined immediately after the weight and density measurements had been made.

Methods

All weight determinations were made on a chemical balance accurate to 0.01 gram. The fish were blotted with paper towels in order to remove excess water before weighing.

Dry weights are given in Table III. The fish were dried in an electric oven for 14 to 20 hours at 95° C. and then for 1 to 2 hours at 105° to 115° C.

TABLE III

Dry weights of Fundulus heteroclitus

Date 1947	Chloride in water m.eq./liter	Sex	Weight grams	Dry weight % of wet weight
Aug. 6	440 (sea water)	♀	2.20	22.0
		♀	2.28	20.8
		♀	1.06	20.8
		♂	1.05	20.9
		♂	2.52	21.0
				Average 21.1
	After 7 hrs. in less than 1 (tap water)	♀	1.48	19.4
		♂	1.40	20.0
				Average 19.7
		% water taken on in 7 hrs. = 6.6		

The method of Lowndes (1938) was employed to determine the density of the fish. The same weighing bottle (51.27 cc.) was used in all determinations. The method is based on the fact that density of fish $= \frac{\text{weight of fish}}{\text{volume of fish}}$. The weight of the fish was obtained both directly and by calculation from weight in water and volume in water. The volume of the fish in sea water was calculated from a comparison of the chloride content of undiluted sea water with the chloride content of the water which had been decanted from the weighing bottle containing the fish and made up to volume with distilled water.

The volume of fish in fresh water was determined colorimetrically at the suggestion of Dr. Hayes. Measurements were made using aquarium water dyed with trypan blue. The volume was determined by colorimetric comparison of water in the volumetric flask (decanted from weighing bottle with fish and made up to volume) and undiluted dyed aquarium water. A Klett colorimeter was used.

Results obtained by the chloride and colorimetric methods were checked by calculating the density from weight in air and weight in water where density of fish $= \frac{\text{weight in air} \times \text{density of water}}{\text{weight in air} - \text{weight in water}}$. These three procedures for determining and calculating densities were carried out on six separate groups of fish in dyed sea water. The standard error for results from the three methods varied from ± 0.0002 to ± 0.0055 in the six analyses made, showing good agreement of the methods.

After weight and density had been determined the swimbladder gas was withdrawn under water using a hypodermic needle and syringe. The gas was immediately expelled into a Krogh micro-gas-analyzer (Krogh, 1908). The volume of gas was estimated in the bulb of the apparatus which was calibrated in tenths of a cubic centimeter. Estimates of small gas volumes were less reliable than those of large volumes. About 0.08 cc. of the gas was analyzed, carbon dioxide being absorbed by one-fourth normal potassium hydroxide, and oxygen by reduced violet chrome alum, as described by Hayes (1939).

Chloride in water samples was determined by Mohr's method. The chloride content of fish tissues was analyzed by means of Van Slyke and Sendroy's micromethod as given by Peters and Van Slyke (1932). All fish were washed under the tap before chloride analyses were made.

Results and Discussion

The results reported here are derived from work done in June and July 1947. Two preliminary series (August 1946; May 1947) were also made for which the data are not complete but which show clearly the same trends indicated in the two series presented in this paper. A fifth series in which fish were transferred to tap water containing calcium carbonate gave similar results, although the loss of salt and gain in weight (water) was not as marked (cf. Weil and Pantin, 1931; Pantin, 1931; Breder, 1933).

Series I. *Response of Fundulus heteroclitus to soft tap water*

The average density of the sea water was 1.019, and of the fish in sea water, 1.023 (± 0.0014 for 16 determinations). This average figure for density of fish

in sea water is derived from density determinations on fish before introduction to tap water of density 0.997. The course of adjustment is presented in Figure 1. Each point in Figures 1A, 1B, and 2 represents an average of two or three determinations, except points at 18 hours, 72 hours, and 7 days where only one analysis was made. In Figure 1C each point indicates a single density determination using one, two, or three fish.

Within 12 hours the density of the fish decreased to 1.001 and remained between 0.995 and 1.006 for the duration of the experiment. The slight rise in density at 24 hours occurred in both series (Figs. 1C, 3) and might reflect the rapid loss of weight (water) at this time (Figs. 1B, 3).

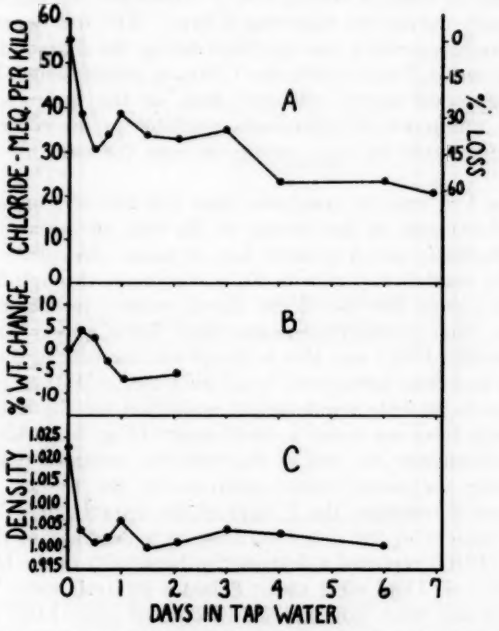

FIGURE 1. Changes in the chloride (A), weight (B), and density (C) in *Fundulus heteroclitus* when transferred directly from sea water to soft tap water.

A rapid gain in weight (4 per cent in 6 hours) follows the introduction of *Fundulus* to fresh water. This phenomenon has been known for some time and Sumner (1905) and Scott (1910) observed an initial gain followed by a loss which persisted throughout the experiment as a result of inanition. The rapid intake of water, probably by way of the gills, is a natural osmotic response to the hypotonic environment. Measurements of the dry weight of two fish at the height of weight gain (7 hours) show a 6.6 per cent increase in water content (Table III). Excretion of water by the kidney may account for the decrease in weight which begins after 8 hours, returning to the initial weight level after about 15 hours and continuing to decrease at a rapid rate for the rest of the first day.

The change in chloride content of the fish is shown in Figure 1A. It should be emphasized that these chloride results apply to the whole fish, and do not reflect the response of any single tissue. By referring to Figures 1A and 1B, it will be seen that an initial dip in the chloride curve corresponds to the curve showing a gain and loss in weight. Since the chloride measurements are calculated on the basis of weight it is likely that the irregularity in the chloride curve during the first day is not real but that chloride is being lost at a constant rate during the rapid gain and loss of water. Sumner (1905) was also aware of this possibility. He found a 25 per cent loss of chloride in *F. heteroclitus* after one day in water having a density of 1.000 or 1.001. The present investigation shows a loss of 30 per cent chloride in one day in water of density 0.997. After the first day chloride continues to be lost gradually during the following 6 days. The fish began to die after the second day, although mortality was not high during the course of the experiments. Of 21 fish in the series, 3 died within the 7 days of experiment. Lack of food may have been a significant factor, although none of the fasting control fish died. Plankton in the unfiltered sea water was available to the controls and no extra demand was being made on their energy as was the case for fish adjusting to fresh water.

From Figure 1 it may be concluded that transfer of *Fundulus* to tap water causes a marked decrease in the density of the fish, an initial increase in weight followed by a decrease, and a gradual loss of tissue chloride. As would be expected in a model osmosis experiment, the water passes through the semipermeable membrane (gills) from the less dense (fresh water) to the more dense (body fluids) solution. Salts (chloride) in the body fluids appear to diffuse out into the water. Sumner (1905) was able to detect this increase in the chloride in fresh water after fish had been introduced from sea water. With regard to water and salts there seems to be little physiological regulation for the first 8 hours by fish introduced directly from sea water to fresh water (Fig. 1). After this period the loss of weight throughout the rest of the first day indicates that the kidneys are probably excreting the excess water taken on by the tissues. Gradual loss of chloride continues throughout the 7 days of the experiment. These changes in salt and water content of the fish contribute to a decrease in the density of the animal. Scott (1910) reported a decrease in the density of the blood of *F. heteroclitus* from 1.0510 to 1.047 after about 8 hours in fresh water. The density of the whole fish at this time, however, has decreased from 1.023 to approximately 1.003 (Fig. 1C).

Adjustment of the density of the fish to fresh water is also assisted by the deposition of gases into the swimbladder, thus making the animal lighter. The function of the swimbladder as an organ for the maintenance of buoyancy in fish is well known, although the exact mechanism whereby fish can separate gases from the blood and deposit them in the swimbladder is still imperfectly understood (Rauther, 1937). The importance of the swimbladder in determining the density of fish is clearly indicated in a paper by Andriaschev (1944) who took density measurements of eight genera of Black Sea fish possessing swimbladders, and eight genera without swimbladders. The range of densities for fish with swimbladders was 1.012–1.021; those without ranged from 1.061 to 1.085.

When *Fundulus* is transferred from sea water directly to tap water, each fish sinks immediately to the bottom for it is heavier than the water. Gas secretion

begins and buoyancy is regained after about 24 hours. The equipment for gas secretion and resorption in *Fundulus* consists of a capillary network and "gas gland." The swimbladder of *Fundulus* is physoclistous, i.e., without an open duct leading to the esophagus. Hence all changes in gas content presumably take place by way of the blood.

An inspection of Figure 2C will show that the volume of gas, measured at barometric pressure, increases about 50 per cent in 24 hours. This increase in gas has the effect of inflating the fish and thus decreasing its density. The greatest part of the secreted gas appears to be oxygen which becomes 175 per cent of normal in 24 hours (Fig. 2A). Carbon dioxide increases somewhat in the first 12 hours,

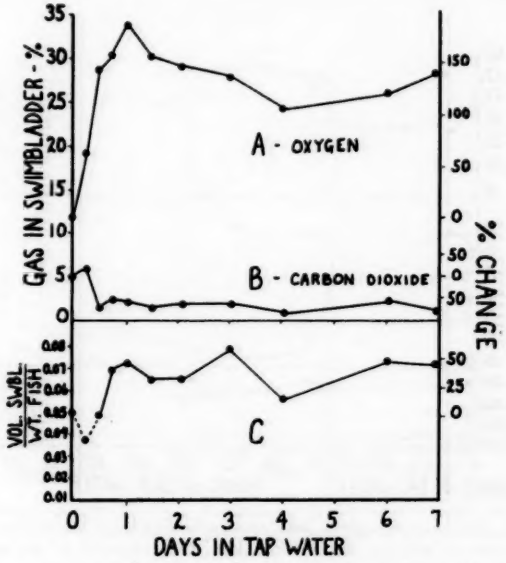

FIGURE 2. Changes in the swimbladder gas of *Fundulus heteroclitus* when transferred directly from sea water to soft tap water.

decreases again before 24 hours, but shows no marked change thereafter (Figs. 2B, 4).

The data pictured in Figure 2 (and Fig. 4) show that *Fundulus* from sea water adjust to the less dense tap water by increasing the volume of gas in the swimbladder. The greatest part of the secreted gas is oxygen.

Series II. *Response of Fundulus heteroclitus to sea water after two days in soft tap water*

A second series of experiments was run to determine the response to sea water of a group of fish whose fresh water history was known. When the fish are returned to sea water they behave like tops and spin around, head down, at an angle

of 45° for about half an hour. The spinning effect is due to the rapid fin movement in an attempt to swim down since they are much lighter than the sea water as a result of the increase in gas volume in fresh water.

The response of the fish on return to sea water is immediate and rapid. The complete history of these fish with respect to change from sea water to fresh water, and the reverse, is presented in Figures 3 and 4. The weight and density measurements on the graphs represent single determinations using two or three individuals. Each point for chloride content and swimbladder gases is the average of results from two fish in the tap water series; in the sea water series, each point represents one fish. The broken line in Figure 3 indicates that the shape of the curve after 24 hours is taken from Figure 1B. In Figure 4 the initial part of the oxygen

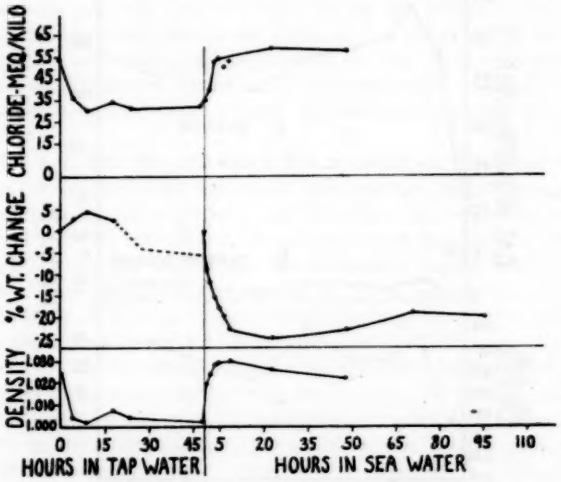

FIGURE 3. Changes in chloride, weight, and density in *Fundulus heteroclitus* transferred from sea water to soft tap water for 2 days, then returned to sea water.

graph is shown by a broken line because of the large difference between the data whose average is presented by the second point (29 per cent and 44 per cent).

The density, chloride, and swimbladder gases return to normal in 4 hours, although it took 12 to 24 hours for the change from sea to fresh water. Loss of weight, due largely to loss of water, appears to be a continuous process reaching a maximum of − 25 per cent. The rate of passage of water out of the fish is three to four times greater than the rate of imbibition when the fish is put into tap water from sea water. The entire adjustment of the fish to the sea water takes place in six hours, or about four times as quickly as the reverse adjustment from sea water to fresh water.

This difference in rate of change of the factors measured might be explained as follows. When the fish is transferred to fresh water the kidneys may start to function immediately, although the effect of their work is not evident until after

8 hours. If water is being excreted by the kidneys during the period of weight gain the net gain would be the difference between water taken on by the tissues and water excreted by the kidneys. When the fish is returned to sea water, however, the water is passing out of the fish both at the gills and kidneys so that the weight (water) loss could be accomplished more rapidly. Some water, however, probably enters with the chloride.

It is difficult to explain the difference in rate of movement of chloride unless the slower loss of chloride in fresh water is due to the functioning of a salt conservation mechanism in the gills. Krogh has shown that several species of fresh water fish can extract salts from fresh water (Krogh, 1939). A similar mechanism may be functioning here so that the fish can in some way regain a little of the chlo-

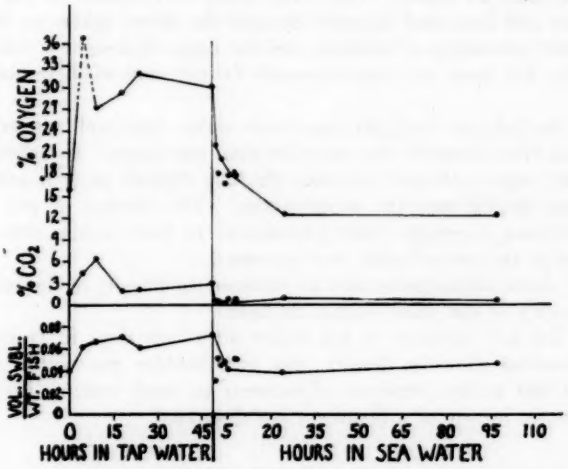

FIGURE 4. Changes in swimbladder gas of *Fundulus heteroclitus* transferred from sea water to soft tap water for 2 days, then returned to sea water.

ride, and so decrease the net chloride loss. When the fish returns to sea water, however, the gradient is such that chloride passes into the fish until the normal salt content is regained.

On the other hand, the gills may not be equally permeable in both directions; or the fish may have some means of controlling movement of water and salts by neural and hormonal activity in a manner similar to that described for the adrenal cortex of dogs (Loeb *et al.*, 1933) and pituitary of frogs (Boyd and Whyte, 1938).

The difference between the time required for the separation of gases from the blood, to increase the gas volume in the swimbladder in fresh water, and that necessary to resorb the gases on returning to sea water might be explained by assuming that the active secretion of gases into the swimbladder would demand more time and energy than the passive resorption of the gases by the blood.

When experiments of the type described in this paper are applied to fish in various stages of acclimatization, a graphic description of the process would then

be available which might facilitate further work on the mechanisms involved in acclimatization to salinity changes.

Summary

1. The adjustment of the killifish, *Fundulus heteroclitus*, to fresh water involves significant changes in the swimbladder gas, chloride content, weight, and density of the fish. Adaptation to fresh water is complete after 24 hours.

2. The gain in weight is only temporary, returning to normal after 18 hours The gain is due to taking on water from the hypotonic medium. The subsequent loss may be the result of kidney excretion and inanition.

3. The chloride decreases markedly during the first 12 hours but some appears to be regained after 24 hours. This irregularity corresponds to the short period of weight gain and loss, and appears because the water taken on by the fish is included in the calculation of chloride on the basis of weight. After 4 days in fresh water the fish have lost approximately 60 per cent of their normal chloride content.

4. When the fish are first put into fresh water they sink immediately to the bottom because fresh water is less buoyant than sea water. In order to adjust to the change and regain normal buoyancy the fish deposit oxygen and, to a lesser degree, carbon dioxide into the swimbladder. The volume of gas measured at barometric pressure is greater after adjustment to fresh water, showing that the amount of gas in the swimbladder has increased.

5. All the above adjustments tend to decrease the density of the fish to approximately the density of the water within 24 hours.

6. When fish are replaced in sea water after two days in fresh water, they regain their normal chloride, density, and swimbladder gas within six hours, or four times as fast as the previous adjustment to fresh water. Possible reasons for this difference in rate of adjustment are discussed.

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A NEW METHOD OF REPRODUCTION IN OBELIA

N. J. BERRILL

McGill University, Montreal

In view of the eminence of *Obelia* as a zoological type enthroned in all text books, and the consequent widespread study of innumerable specimens, it is surprising that there could be an important method of reproduction of this genus so far unreported.

The observations recorded here were made in the course of extensive investigation of growth and form in *Obelia*, involving day by day study of specific colony sites through the summer of 1947 at Boothbay Harbor. Temperature changes were followed closely since colonies of *Obelia* and other hydroids fluctuated greatly, disappearing and reappearing as temperatures rose and fell markedly above and below 20° C. Three species were studied, all associated with one float, *Obelia articulata, O. geniculata* and *O. longissimus.*

For nearly two months of excessively high temperatures during July and August, no colonies could be found. With the onset of offshore winds, the warm surface water blew out of the bay, to be replaced by bottom water 8 to 10 degrees colder. With this lowering of the temperature, small *Obelia* colonies appeared in relatively large numbers. Calm weather with no wind except the daily inshore breeze allowed the surface bay waters to warm up again to about 21° C. for a few days, followed by a slow fall to lower temperatures. The growth or reproductive procedures described here were responses to these changes.

Obelia articulata

This species grew attached to laminaria. Colonies are relatively small but well branched, and in general are intermediate in character between the single unbranched stems of *O. geniculata* and the enormously long and branching colonies of *O. longissimus.* The intermediate character is again shown in the distribution of the gonangia. In *O. geniculata* they grow out from the angles made by the hydranths and the stem. In *O. longissimus* they appear at the angles made by hydranths with lateral branches but only at the basal region of a colony after it has already become massive. In *O. articulata* they appear when the colony is small, but at angles between hydranths and secondary branches, not in connection with the main stem. Similarly the growing tip of the main stem in colonies of all three species varies in series. It is essentially a stolonic type of growth like that of the creeping stolons. In *O. longissimus* it grows rapidly and vigorously, giving off secondary stolonic outgrowths regularly at a certain distance from the tip, and these behave in much the same way. Hydranths are mainly tertiary outgrowths, at least. In *O. geniculata* terminal stolonic growth is very limited, lateral branches are not formed, and the tip itself usually differentiates into a hydranth. *O. articulata* lies between.

In any species a rise in temperature, especially when in excess of 20° C., tends to maintain or promote stolonic growth at the end of a stem or branch of any order and conversely to inhibit hydranth differentiation. The outgrowths capable of responding in one way or another to different temperature conditions may be of varying origin. They may be the terminal tips of the main stem and secondary branches, tips of branches of a more subsidiary order, or the tips of presumptive gonangia at stem or branch angles.

A marked rise in temperature results in prolonged growth of a stolonic character in the first two cases, the long slender branches thus formed remaining an integral part of the colony, even though they may in no way contribute to its welfare. In the third case, those normally destined to become gonangia, the reaction is different.

A gonangium in its earliest recognizable stage is shown in Figure 1A, growing from the non-annulated region immediately below a hydranth. It consists of an outgrowth with several annulations, terminating in a relatively large bulb with a short central cone and wide shoulders. This persists and grows as the distal cap of the gonangium. In the same figure on the same scale are shown outgrowths from homologous locations, but from colonies subject to higher temperatures. Annulated growth, instead of stopping after two or three annulations and forming the wide gonangium rudiment, continues until ten or a dozen shallow annulations have occurred. The final surge corresponding to the establishment of the gonangium leads instead to the formation of a massive elongate stolonic structure with no further trace of annulations. It is similar to the terminal stolonic growth at the ends of branches, but with two differences, it is much more massive and of greater girth, and is so vigorous that the stem uniting it to its point of origin becomes attenuated to the point of rupture (Fig. 1B). Distally each such mass grows rapidly, while it resorbs correspondingly at the proximal end. The separation usually occurs at the region where the annulated growth transforms into a steady surge (Fig. 1C), the part left attached to the colony retracting proximally as the tension is relaxed, while the congested terminal units slide out of the thin but wide perisarcal tube to float freely in the surrounding water.

The question that arises at once is whether this is a normal process or a response to the disturbance of collection and subsequent examination. Obelia and similar hydroids are notoriously susceptible and it is a common experience to find hydranths and other terminals in process of regression with distal parts of the coenosarc often isolated within the perisarc from the main body. This possibility was considered immediately, and the following is the evidence that the process is a normal one for the sea temperature prevailing at the time. Colonies picked off the laminaria and dropped into formalin within a few seconds of emergence from the water exhibited the phenomenon to as great a degree as any. The colonies under live examination were fresh, had hydranths with active tentacles and manubrium, possessed hydranth buds that progressed normally to complete development, and showed no trace of resorption at any of the terminals.

Small colonies left standing in finger bowls liberated literally hundreds of gonangial terminals overnight and were still in active process the following day. Lastly, there is the evidence that they possess a useful function. In the first place, fragments of ordinary terminals of equivalent length but smaller diameter can reattach and survive for a week or two. They do not develop hydranths unless

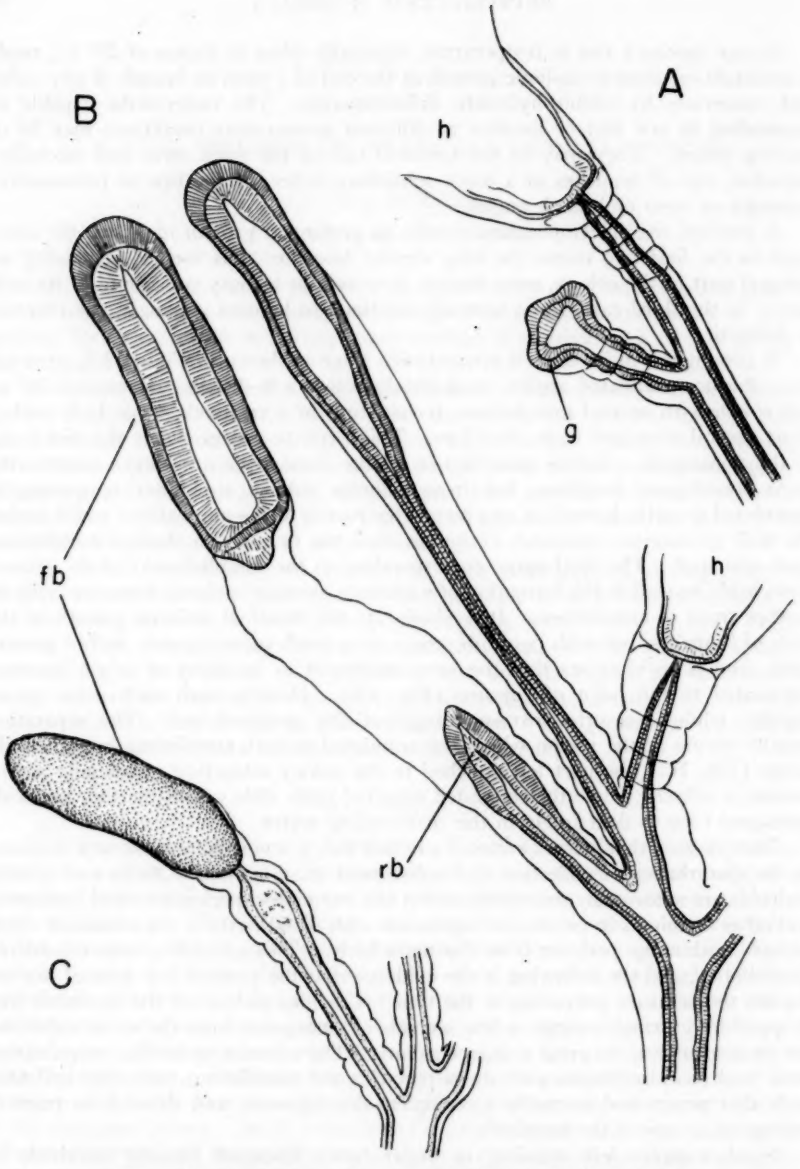


FIGURE 1. Production of free gonangial buds in *Obelia articulata*.

A. Hydranth stalk with young gonangium. B. Hydranth stalk with gonangial buds in process of formation and liberation. C. Gonangial bud showing constriction at junction of annulated and non-annulated regions. *fb*, free buds; *g*, young gonangium; *h*, hydranth; *rb*, retracted stalk after liberation of bud.

several times as long. The isolated gonangial terminals on the other hand become attached to a solid substratum immediately upon contact. After about 12 hours, each fragment is about twice its original length and half its girth. The original perisarc, however, indicates that most of the tissue is now the result of new distal growth and proximal resorption (Fig. 2B). At the same time an annulated up-

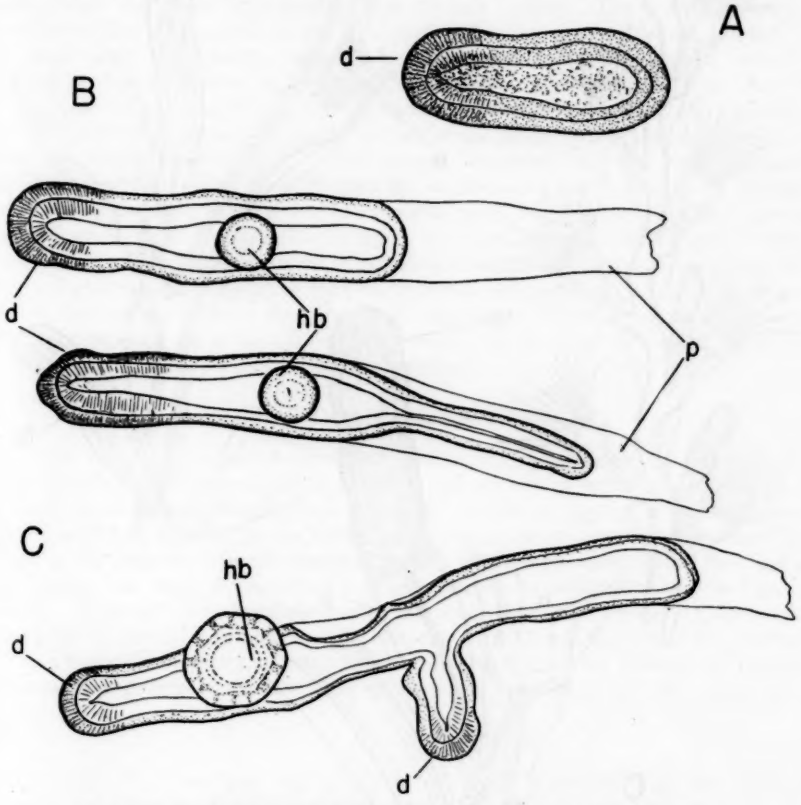


FIGURE 2. Development of free gonangial bud of *Obelia articulata*.

A. Bud at time of attachment. B. Twelve hours later with hydranth buds growing vertically and empty perisarc indicating extent of proximal resorption and distal growth. C. Twenty-four hours after attachment, with hydranth bud at tentacle rudiment stage, and with secondary distal outgrowth. *d*, distal growing region; *hb*, hydranth bud.

growth from the middle of the fragment indicates a developing hydranth. In Figure 2C a fragment is shown typical of the condition 24 hours after liberation. A lateral creeping stolonic terminal has started, while the hydranth has progressed to the tentacle rudiment stage. In the great majority of the liberated fragments, functional hydranths were present on the second day and new colonies thus started.

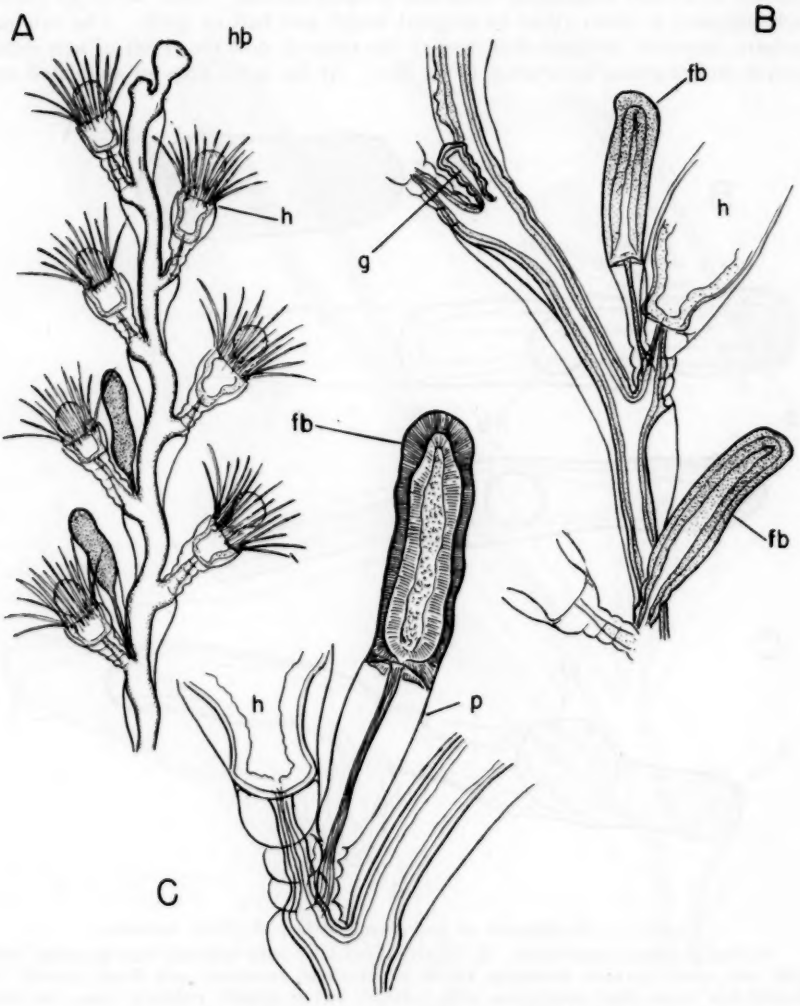


FIGURE 3. Production of gonangial buds in *Obelia geniculata*.

A. Complete sprig with two advanced gonangial buds. B. Part of stem showing young gonangium and two stages in production of gonangial buds. C. Bud showing sharp demarkation between massive presumptive free bud and attenuated proximal stalk. *fb*, presumptive free bud; *g*, gonangium; *h*, hydranth; *p*, perisarc.

The immediate developmental capacities are accordingly somewhat superior to those of the average planula.

Obelia geniculata

Colonies of *Obelia geniculata* were collected a day later than those of *O. articulata*, when the water temperature was already falling. The great majority of stems had the appearance shown in Figure 3A. No gonangia were present, but in their place were large congested terminals similar to those of *O. articulata*. Detail as seen in Figure 3C indicates that the process is essentially the same, the distal part of the massive stolonic outgrowth growing rapidly at the expense of proximal tissue. In fact the proximal half of the outgrowth becomes so attenuated that the lumen is obliterated. While actual separation was not observed in this species, continued distal growth after the occlusion of the lumen must inevitably result in a break in the attenuated proximal part. In Figure 3B two stages are shown, one with an attenuated stalk and a younger stage with wide lumen throughout. A third, the most anterior, is a younger outgrowth and is developing into a typical gonangium, suggesting that the external temperature had already dropped below the critical value at the time of its initiation. At the same time it indicates the relative scale of the two forms of growth from the hydranth-stem angles, and the comparative massiveness of the high-temperatures' outgrowth.

Obelia longissima

This species is included merely as a basis for comparison. It is typified by the very extensive growth of the primary and secondary terminals, leading to the formation of relatively enormous colonies. Gonangia appear in secondary and other angles at the base of the colony only after it has attained a fairly large size. During the warmer summer months growth is directed mainly into the vigorous terminals, and there appears to be little tendency to form gonangial outgrowths at all. They are most abundant during late winter and early spring. Consequently the type of asexual reproduction just described for *O. articulata* and *O. geniculata* is here probably of very rare occurrence, if it occurs at all, for without the initiation of gonangial outgrowths of any kind, no response in either direction is possible.

Summary

A method of asexual reproduction previously unrecorded is described for *Obelia articulata* and *Obelia geniculata*.

When water temperatures markedly exceed about 20° C. presumptive gonangial outgrowths continue growth as massive stolonic terminals that rapidly constrict off, leave the colony and settle elsewhere to establish new colonies in large numbers. In size and potentiality these reproductive units somewhat exceed those of typical campanulid planulae.

TOOTH SUCCESSION IN THE SMOOTH DOGFISH, MUSTELUS CANIS

JOHN D. IFFT
Simmons College, Boston

AND

DONALD J. ZINN
Rhode Island State College

The arrangement of the teeth of sharks in a series of rows is well known. In some species, such as tiger sharks and sand sharks, with large conical teeth, newly formed teeth appear to be formed in the back rows while older teeth are in front. This impression led Owen in 1866 to state, ". . . the whole phalanx of their numerous teeth is ever marching slowly forwards in rotary progress over the alveolar border of the jaw, the teeth being successively cast off as they reach the outer margin, and new teeth rising from the mucous membrane behind the rear rank of the phalanx." Owen's theory of tooth replacement in sharks is the commonly accepted one today and is found in most comparative anatomy texts. This theory apparently was based only on morphological evidence without experimental proof; a search of the literature has failed to reveal reports of any experiments testing the theory. However, the morphological evidence is quite convincing and accounts for the general acceptance of the theory.

Within recent years Owen's hypothesis has been challenged by Cawston in a series of papers (1939; 1940a, b, c; 1941a, b, c; 1944; 1945). He has doubted that sharks shed their teeth but if they do he denies the possibility of replacement occurring by the forward movement of teeth from the rear. That sharks shed their teeth is confirmed by Breder (1942) who noticed the sloughing of teeth by sand sharks (*Carcharias littoralis*) in the tanks at the New York Aquarium. Whether the lost teeth are replaced and the manner of this replacement if it occurs apparently has not been observed. It is the purpose of this investigation to inquire experimentally into the question of polyphyodonty in selachians.

Materials and Methods

It was thought at the beginning of this work at Woods Hole, Massachusetts, that both the spiny dogfish (*Squalus acanthias*), and the smooth dogfish (*Mustelus canis*) could be used. However, the spiny dogfish would not live in the aquaria. Perhaps this may be caused by normal summer salt water temperature in Woods Hole being lethal for the spiny dogfish but not for the smooth dogfish. This was suggested by William Schroeder, Jr., of the Woods Hole Oceanographic Institute who in conversation with the authors pointed out the coastwise migrations of the spiny dogfish paralleling temperature isotherms.

Since *Squalus* proved unsatisfactory, *Mustelus canis*, collected at Woods Hole, Massachusetts, were used in these experiments. A total of 23 adult animals were

used, one group of 12 in the summer of 1946 and a second group of 11 in the summer of 1947. The animals ranged in size from 14½" to 39" with the majority being over 24" in length; 11 were males, 12 females. They were kept in a large paraffin-lined cement tank supplied with running sea water and were fed every other day on chopped fish.

The dogfish were anesthetized by cooling in ice water according to the method of Parker (1937) and a varying number of teeth, as described below, were removed with forceps from the lower jaws. In order to follow the movements of the remaining teeth they were marked with silver nitrate solution precipitated with stannous chloride. While the stain subsequently was worn away from the surface of the teeth, sufficient amounts remained on the sides of the teeth to mark them adequately. This species has pavement teeth, somewhat diamond-shaped and arranged in compact rows (see Fig. 3). Sections were made of the jaws using both paraffin and celloidin technics following decalcification. Mallory's stain as well as haemotoxylin and borax-carmine was used.

We wish to thank the Woods Hole Oceanographic Institute and the Marine Biological Laboratory for the use of their facilities.

Experiments and Observations

The preliminary experiments were designed to determine if tooth replacement occurs in *Mustelus*. For this purpose 12 animals were divided into four groups. In the first group of three animals, six teeth of the first row in the mid-line of the lower jaw were removed. These animals died six, eight, and 11 days respectively after the operation. The cause of death was not ascertained although it probably was not the result of the operation since one of the unoperated controls died during the same period. The teeth were not replaced in this period. Serial sagittal sections at 10 μ revealed no change had taken place and the jaws presented the usual appearance with tooth buds in successive stages of development posterior to the area of the erupted teeth.

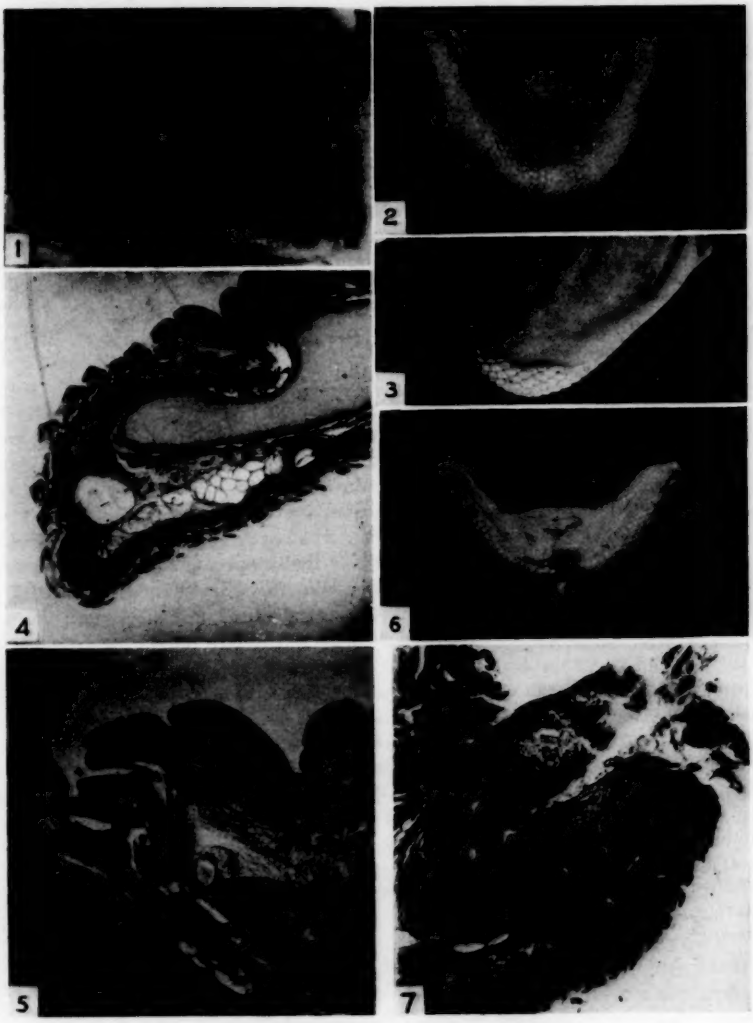
The second group contained four animals from each of which 22 teeth were extracted from a triangular area, five rows deep; the apex of the triangle pointed posteriorly. Figure 1 is a photograph of a jaw of this group. Two of the fish died before replacement occurred, after eight and 12 days respectively. The remaining two replaced the teeth within 50 days. Figures 2 and 3 are photographs of the jaw of one of these latter fish. It can be seen that the replaced teeth are arranged in the normal pattern. Sections of these jaws also were normal in appearance (Fig. 4).

The third group of three animals had the first row of teeth removed. Two died on the following day but the third had replaced the teeth when examined 93 days later. The rate of replacement was not obtained for this animal.

The fourth group consisted of the two control animals. Both were anesthetized by cooling but were not operated upon. One died the following day, the other in 18 days. The cause of death was not determined although the method of anesthetizing might have been a contributory cause.

The second series of experiments were designed to discover the manner in which the tooth replacement occurred. The 11 dogfish of this series were divided into three groups. In the first group of four, each of the fish had 12 teeth in all ex-

PLATE I



tracted from the anterior first two rows in the center section of the lower jaw. The remainder of the teeth with the exception of the two first rows lateral to the extracted area were marked with silver nitrate. One animal died on the ninth day and no change in the teeth was found. The other three were examined 25 days later and all had replaced the extracted teeth with teeth bearing silver nitrate marks. In addition, the teeth lateral to the extracted area, previously unmarked, now were replaced by teeth bearing silver nitrate markings. This would seem to indicate, therefore, that within the 25-day period, two rows of teeth moved forward and replaced the former first two rows.

The second group of this series consisted of five animals in which either two, three, or four rows in the center section were removed, and the tooth-bud area back of the region from which the teeth had been extracted, was cauterized with an electric cautery. Four of these animals died in three, five, 12 and 13 days respectively. The remaining animal of the group lived and was killed 25 days later. In the three cauterized dogfish living 12, 13, and 25 days the tooth area in front of the region cauterized was disorganized: many teeth in addition to those extracted had fallen out and only a few scattered teeth remained in the center area. Figure 6 is a photograph of the jaw of one of these fish. No replacement of teeth had occurred in any of this group including the animal killed after 25 days. A section (Fig. 7) from this latter dogfish taken through the cauterized area and the region anterior to it shows the drastic disorganization resulting from the cauterization. The tooth buds were destroyed and parts of the jaw cartilage degenerated. The oral epithelium and underlying connective tissue appeared to be sloughing off.

The third group contained two animals in which all but the first two rows of teeth were marked with silver nitrate but no teeth were extracted. Both of these fish died six days later; there were no observable changes in the teeth.

Certain general observations of the teeth were made. It was found that the first or outermost row of teeth was irregular while the preceding rows are quite regular. This would seem to indicate that the teeth are normally lost singly from the first row as has been observed in other species. Great regularity was observed in the posterior rows and in the animals examined there were no indications of tooth-loss except in the first row. The number of exposed rows of teeth varied from eight to 11. No sexual differences in the teeth were seen. The arrangement of the teeth in the upper jaws appeared to be similar to that of the lower jaws.

PLATE I

FIGURE 1. View of jaws of dogfish showing triangular area in center of lower jaw from which teeth have been extracted. About one-third natural size.

FIGURE 2. Dorsal view of jaw of animal in Figure 1 fifty days after removal of teeth showing the complete replacement of the teeth. About one-third natural size.

FIGURE 3. Ventral view of jaw in Figure 2. About one-third natural size.

FIGURE 4. A sagittal section at 10 microns of the jaw seen in Figure 2. Tooth buds can be seen back of the erupted teeth. About × 10.

FIGURE 5. A view of the tooth bud area from Figure 4. About × 33.

FIGURE 6. A dorsal view of a jaw in which 4 rows of teeth were removed in the center section and the tooth buds back of this region were cauterized. No replacement had occurred after 25 days. About one-third natural size.

FIGURE 7. A sagittal section at 10 microns of the jaw seen in Figure 6, showing the disorganization resulting from the cauterization. About × 10.

Tooth-bud areas were never found except behind the tooth-bearing region. Figure 5 is a photograph of the tooth-bud area. The tooth buds can be seen to be progressively larger and more mature in a postero-anterior direction. Particular care was taken to search for buds underlying the outermost rows but none were found. It would appear, therefore, that the only source of new teeth are these buds back of the erupted tooth area.

Conclusions and Discussions

From the experiments described above it seems apparent that in *Mustelus canis* teeth can be replaced and that this replacement occurs in the manner hypothecated by Owen; that is, by the moving forward of the teeth from the rear. The fact that marked teeth from posterior areas were seen later to occupy areas where teeth had been removed seems conclusive evidence in favor of Owen's view. It is not certain from the experiments what the normal rate of replacement is since the animals which were to have been used to test this point died before such information could be obtained. However, the rate of replacement in the operated animals was quite rapid, being approximately of the order of one row replaced in ten to twelve days.

The experiment in which the tooth buds back of the center area of the jaw were removed by cautery was done to determine whether replacement occurred in the absence of the posterior tooth buds. In the one surviving animal replacement had not taken place although in the same length of time non-cauterized dogfish did replace teeth. While the experiment apparently bears out the role of the posterior tooth buds in replacement it may be criticized on the ground that the unexpected general disorganization and degeneration resulting from the cauterization would prevent replacement from any source. However, even if this experiment is omitted from consideration, there is sufficient evidence from the other experiments to support the contention that Owen's hypothesis is correct.

From a study of Cawston's papers it would appear that his views are based on gross examination only and without a study of histological sections. Otherwise it is difficult to account for his statement (1941a): "New tooth formation behind the normal number of rows of teeth in species of shark has never been observed, though dental germs should be present if the alleged replacement of teeth by revolving of the gum forwards ever occurred in adult specimens." In the same paper he also states: "At the anterior border of the teeth of *Mustelus canis* (Mitch.) one sees round or oval dental germs in process of development into the flattened closely set teeth of the adult, which reveal the characteristic wrinkled surface very early." As we have noted earlier, and as can be seen from the photographs of the sections (Figs. 4, 5), tooth buds are found back of the erupted teeth and are not found in the front region of the jaw. There is no evidence that new teeth are being formed in the front row of *Mustelus*.

In a later paper (1944), Cawston states that there is no provision for replacement of lost teeth in selachians and that growth may continue throughout life. In earlier papers (1939, 1941a) he considers that a tooth is renewed at the site where one is lost. He considers that this replacement obtains by vertical succession (1941b). Unless we are misinterpreting the statements it would appear that Cawston's viewpoint has changed from a possibility of vertical succession in tooth replacement to the hypothesis that no replacement of any type occurs.

Other observers besides Owen have concluded by studying the morphology of the jaw that replacement occurs by the forward movement of the back teeth. For example, Budker (1938) states: "Lorsque la dent est tombée, une autre, dite 'dent de remplacement' et provenant des rangées de remplacement disposées derriere les rangées fonctionelles, vient prendre sa place." This author also observed that tooth buds did not develop at the site of the lost tooth.

The cause of the falling-out of the teeth was also studied by Budker in various species such as *Scyliorhinus canicula*. He accounted for this loss by the destruction of the dentinal basal plates which anchor the tooth in the underlying connective tissue by specialized cells similar to osteoclasts which cells also reduce the dentine of the older tooth as a whole. Benzer (1944), on the other hand, reports that the dentine of *Mustelus* grows progressively thicker in older teeth. He did not note that the dentine was later destroyed.

The jaws of ten other species of sharks were examined by the authors through the courtesy of Mr. Schroeder at the Museum of Comparative Zoology at Harvard University. Included in the group were three species of the Port Jackson shark (*Cestracion* or *Heterodontus*) which have pointed biting teeth in front and flat crushing teeth in the remainder of the jaw. It was observed, however, that the teeth in any section of the jaw are the same in an antero-posterior direction and consequently could be replaced in the manner described for *Mustelus*. No morphological indications were found in any of the other species examined contradicting Owen's hypothesis.

Summary

1. Twenty-two teeth extracted in a triangular area five rows deep from the front of the tooth-bearing region of the lower jaw of *Mustelus canis* were replaced within 50 days.

2. Marking of the posterior teeth with silver nitrate indicated that extracted teeth were replaced from behind by these marked teeth. The replacement rate was approximately one row in 10 to 12 days.

3. Tooth buds were found only back of the erupted teeth and never elsewhere.

4. Destruction of the tooth buds by cautery prevented replacement.

5. It is concluded that Owen's hypothesis of the replacement of sharks' teeth by the forward movement of the posterior teeth is correct and that Cawston's objections to the theory are not tenable.

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POSTEMBRYONIC GROWTH CHANGES IN THE ISOPOD PENTIDOTEA RESECATA (STIMPSON) WITH REMARKS ON THEIR TAXONOMIC SIGNIFICANCE

ROBERT J. MENZIES AND RICHARD J. WAIDZUNAS

Pacific Marine Station, College of The Pacific, Dillon Beach, California

Introduction

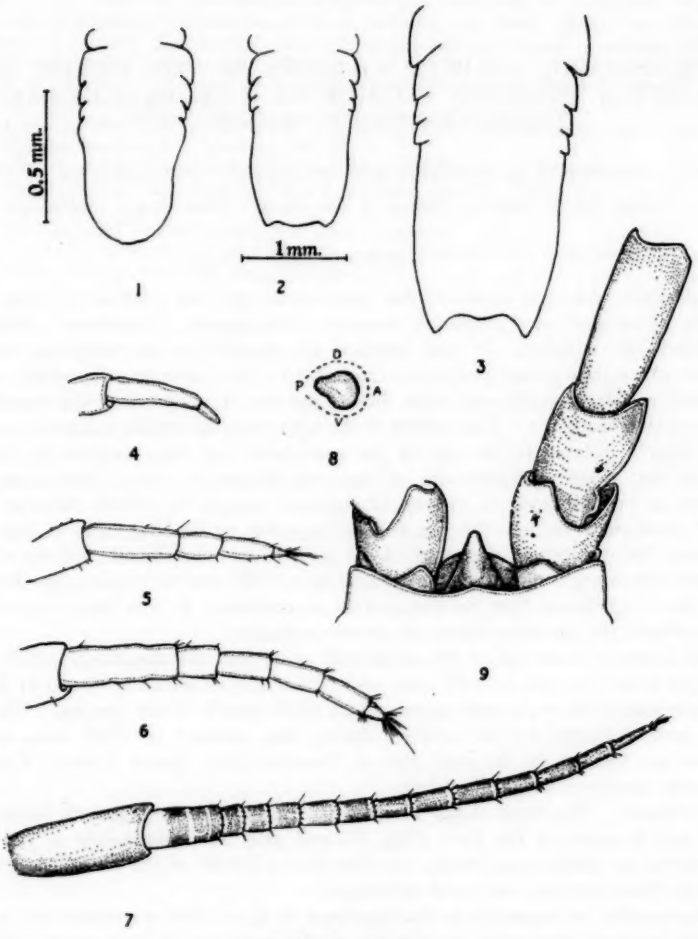
This study was the result of the observation that the number of setae of the seventh peraeopod of *Pentidotea resecata* (Stimpson) (Valvifera: Idotheidae) was markedly variable. It was decided to conduct an investigation including features other than peraeopod setal number, in order to determine which features remained relatively stable and were thus of specific significance in the classification of the marine idotheids. The nature of the variations in certain features was found to be directly related to the size of the specimens, and thus to growth; and it is believed that these variations are of basic significance to isopod taxonomy. The features at present used to distinguish marine isopods of North America of the family Idotheidae include the number of segments of the flagellum of the second antennae, the number of segments of the palp of the maxilliped, and the shape of the posterior margin of the telson (Richardson, 1905 and references, pp. 346–408). It is most significant that, in the species investigated, it was these features that demonstrated the greatest degree of growth variation.

The material consisted of ten adult and seven juvenile specimens which ranged in length from 5.2 mm. to 43.0 mm. and of ten far advanced embryos of 2.2 mm. length, removed from the marsupium of an adult female of the species. The specimens were collected by the writers during the summer of 1947 from eelgrass, *Zostera* sp., located on the sand flats of Tomales Bay, Marin County, California, where the species is fairly abundant.

The head. The most stable features of the head during growth included the shape and location of the eyes (Fig. 8) and also the relationship of the frontal laminae to one another and to the anterior-dorsal border of the head. In embryos, however, these features were not developed.

The number of segments to the flagellum of the second antennae was found to increase in direct proportion to the size of the specimen at least until adult status was reached. This phenomenon was observed in part by Hale (1946, Fig. 19, p. 193) in his description of *Antarcturus horridus* Tattersall (Arcturidae). Embryos 2.2 mm. long had two segments to the flagellum of the second antenna. In a specimen of 5.2 mm. and one of 6.0 mm. length the number of segments was four. In two specimens of 8 mm. length, one of 9 mm. length and in one specimen of 10 mm. length the number of segments was seven; while in one specimen of 9.5 mm. length the number of segments was eight. Even among the larger specimens the number of segments of the flagellum was observed to vary considerably. The length of the flagellum appears to be proportional to the body length in the juvenile

PLATE I



Pentidotea resecata

FIGURE 1. Abdomen, dorsal view, embryo, 2.2 mm. length. Magnification as indicated.
FIGURE 2. Abdomen, dorsal view, juvenile, 5.2 mm. length. Magnification as indicated.
FIGURE 3. Abdomen, dorsal view, small adult male, 9.5 mm. length. Magnification as per Figure 2.
FIGURE 4. Flagellum second antenna, left, embryo, 2.2 mm. length. Magnification as per Figure 1.
FIGURE 5. Flagellum second antenna, left, juvenile, 5.2 mm. length. Magnification as per Figure 1.
FIGURE 6. Flagellum second antenna, left, small adult male, 9.5 mm. length. Magnification as per Figure 1.

specimens of *P. resecata,* while in the adult specimens the length of the flagellum varied.

In specimens of 6.5 mm. and below, the palp of the maxilliped was four jointed. More developed specimens had a maxilliped palp of five segments. At present the only character used to distinguish the genus *Pentidotea* (Richardson, 1905, p. 368) from *Idothea* (ibid., p. 356) is the presence of a five-jointed palp in the former and of a four-jointed palp in the latter (see also Light, 1941, p. 87). It seems evident to the writers that either *Pentidotea* must be considered a synonym of *Idothea* or that the generic differences must be redefined.

The increase in setae on the tip of the endopodite of the maxilliped was found to be correlated directly with the size of the specimen (compare Fig. 17 and Fig. 19). The same was true of the "hairiness" of the median border of the palp of the maxilliped. Only the presence of a single coupling-hook on the median border of each maxilliped was found to be constant (Figs. 17 and 19, "x").

The perion. The relationship in length of the lateral border of the epimeral segments of the perion to the length of the lateral border of the perion segments themselves appeared to be constant, yet measurements made at the second and third perion segments showed considerable variation. This variation did not correlate directly with a size increase of the specimens and the writers believe that the difficulty in obtaining accurate measurements of these structures accounts for the irregularity. Observations indicate that the seventh perion segment remained in an undeveloped state in juvenile animals as large as 5.2 mm. Its retarded development was best indicated on embryo specimens. The general narrow shape of the animal was maintained in animals of all sizes. Ovigerous specimens showed a distinct lateral widening of the segments of the perion concerned with the marsupium development.

The peraeopod. The number of setae on the seventh peraeopod was examined carefully in the hope that a very definite non-variable structural feature could be found. The number of setae on the ventral surface of the propodus was observed to be directly proportional to the size of the animal. Three distinct types of setae are discernible; a "saw-toothed" seta (Fig. 11), a "file-toothed" seta (Fig. 10), and a "simple" seta (Fig. 12). A once specialized seta observed on the propodus of the seventh peraeopod of a small animal was always found without modification, other than increase in size, on the propodus of the seventh peraeopod of a larger animal. The "saw-toothed" type seta was constant in number and location. The "file-toothed" type seta was more numerous on the propodus of the seventh peraeopod of larger specimens and the same is true of the "simple" type setae which most frequently surrounded the "file-toothed" seta. Evidence indicates that the "file-toothed" seta is developed, in part at least, from one of the "simple" type.

It was found that the seventh peraeopod was weakly developed in very small specimens (below 6.0 mm. length); whereas the first to the sixth peraeopods were

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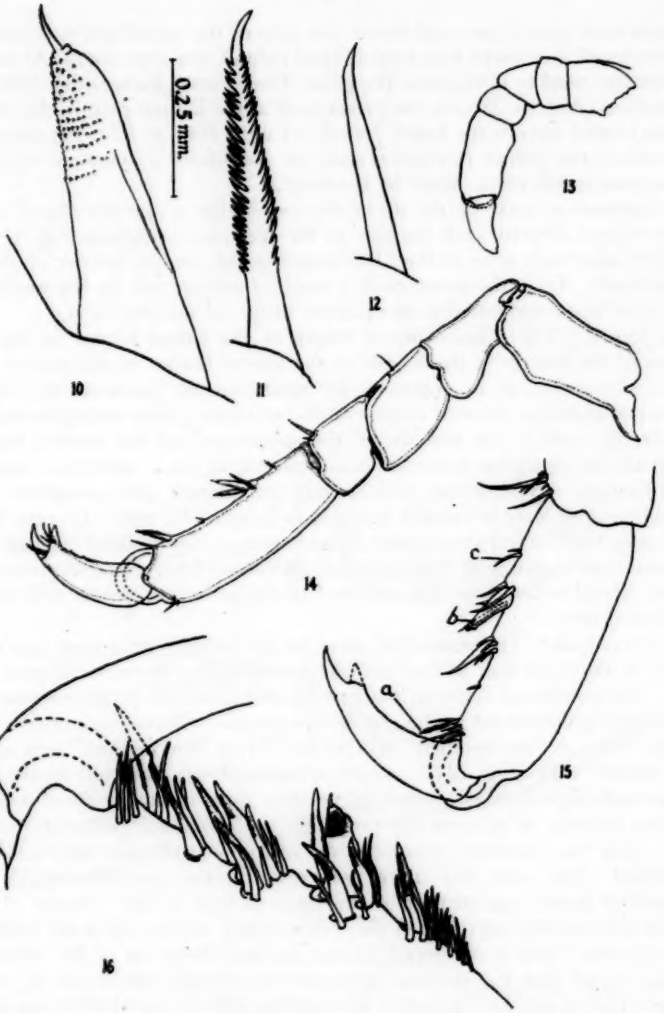
FIGURE 7. Flagellum second antenna, left, adult male, 20.5 mm. length. Magnification as per Figure 2.

FIGURE 8. Eye, left, lateral view, adult male, 20.5 mm. length; "P" posterior, "A" anterior, "D" dorsal, "V" ventral. Magnification as per Figure 2.

FIGURE 9. Anterior-dorsal border and first two frontal laminae, adult male, 20.5 mm. length. Magnification as per Figure 2.

PLATE II



Pentidotea resecata

FIGURE 10. File-toothed seta of ventral surface of propodus, see "b" of Figure 15. Magnification as indicated.

FIGURE 11. Saw-toothed seta of ventral surface of propodus, see "a" of Figure 15. Magnification as per Figure 10.

FIGURE 12. Simple seta of ventral surface of propodus, see "c" of Figure 15. Magnification as per Figure 10.

well developed. The retardation in the development of the seventh peraeopod has been observed in other isopods (Sømme, 1940, Limnoriidae, p. 158; Faxon, 1882, Asellidae, pl. vi, Fig. 19; Hult, 1941, Parasellidae, p. 39).

The telson. The concavity of the posterior margin of the telson has been regarded as the most diagnostic and key feature of this species. Actually, however, the margin was found by the writers to change very gradually from one with a convex posterior border in the embryo (Fig. 1) to a slightly concave margin in specimens of 6.5 mm. in length (Fig. 2); until, when the adult condition is reached (Fig. 20), the concavity is most developed.

The uropod conforms to the shape of the telson and therefore varies in accordance with its size and shape. The number of movable setae at the lateral articular distal border of the penultimate uropod segment varied in number from one to two regardless of size or sex of the specimen.

It is evident from the above observations that very young specimens of *Pentidotea resecata* might very well be placed in the genus *Idothea* and considered new to science by an investigator unaware of the developmental nature of the maxilliped palp. Such would be true at least as long as the two genera *Idothea* and *Pentidotea* remain so briefly designated. Indeed one writer, Fee (1926, p. 18, Fig. 12) did just that in describing *Idothea rufescens* from specimens which apparently are juvenile specimens of *Pentidotea resecata* (Stimpson).

The suggestion of course from the above is that authors of new species of Idotheid genera (as well as isopods in general) not only indicate the measurements of the types but also give measurements of all specimens figured or described in the text. To date such a procedure has been followed by only a very limited number of workers and even then without any marked degree of consistency.

Summary

In an attempt to find constant characteristics which may be relied upon as specifically diagnostic in the marine isopod *Pentidotea resecata,* the following features proved to be especially significant constant features regardless of the size of the specimen: (1) structural interrelationships of the frontal laminae, (2) epimeral plate length in relation to the length of the lateral border of the corresponding perion segment, (3) the character of the setation and certain features of peraeopod morphology, (4) general body shape.

Features showing numerical increase which was found to be directly proportional to the size of the animal and thus believed to be of very limited taxonomic utility include: (1) number of segments to the flagellum of the second antennae, (2) number of segments to the palp of the maxilliped, (3) number of setae of the maxilliped and of the peraeopods.

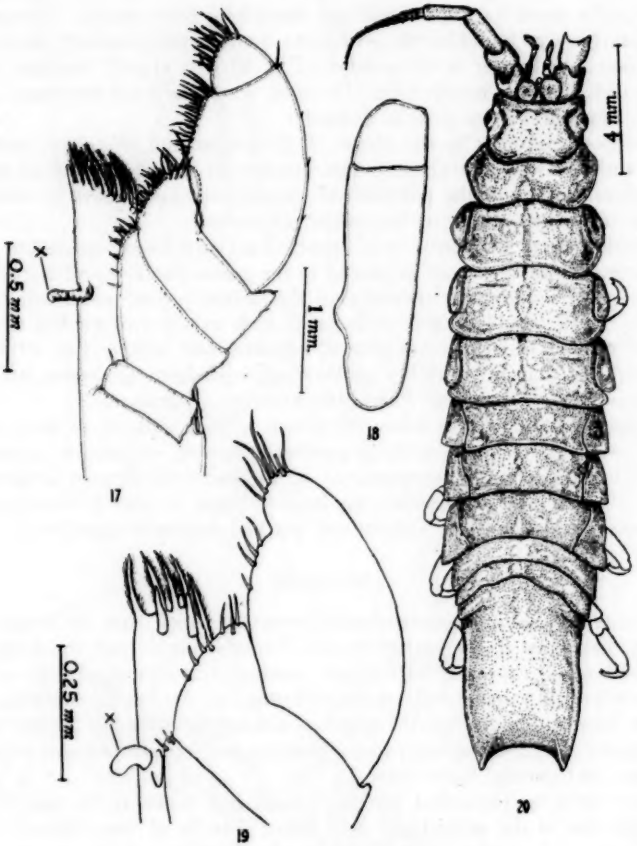
Figure 13. Seventh peraeopod, right, juvenile, below 6.0 mm. length. Magnification as per Figure 1.

Figure 14. Seventh peraeopod, left, juvenile, above 6.0 mm. length. Magnification as per Figure 1.

Figure 15. Propodus and dactylus of seventh peraeopod, small adult male, 9.5 mm. length. Magnification as per Figure 1.

Figure 16. Ventral border of propodus of seventh peraeopod of large adult male, 20.5 mm. length. Magnification as per Figure 1.

PLATE III



Pentidotea resecata

Magnification as indicated

FIGURE 17. Maxilliped, left, small adult male, 9.5 mm. length; "x" is the coupling-hook.
FIGURE 18. Uropod, left, small adult male, 9.5 mm. length.
FIGURE 19. Maxilliped, left, juvenile, 5.2 mm. length; "x" is the coupling-hook.
FIGURE 20. Dorsal view, adult male, 20.5 mm. length.

It would seem to be necessary, in view of the findings, to reexamine the status of species and genera which owe their existence exclusively or in part to characteristics here shown, in this species at least, to be variable in different age groups.

One species *Idothea rufescens* Fee, apparently based on immature specimens, is considered a synonym of *Pentidotea resecata* (Stimpson).

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THE UTILIZATION OF SUGARS AND OTHER SUBSTANCES BY DROSOPHILA

CHARLES C. HASSETT

From the Medical Division, Army Chemical Corps, Army Chemical Center, Maryland

Studies have been made of the use of carbohydrates and other food material by several insects, e.g. the honey-bee (Bertholf, 1927; Phillips, 1927; Vogel, 1931), the blow-fly (Fraenkel, 1936, 1940), the Mexican fruit fly, *Anastrepha ludens* (Baker et al., 1944), and a number of others. The reviews of Trager, 1941 and 1947, and Uvarov, 1928, furnish extensive references. *Drosophila melanogaster* seems, however, to have escaped attention in this connection heretofore. Experiments have now been made on the ability of this fly to utilize a large number of carbohydrates and related compounds, as well as some substances of other classes. In addition, an estimate of the relative nutritional efficiency of these substances has been made.

Material and Methods

Adults. To rear flies for these tests, the standard corn meal, agar, and sugar medium, in half-pint milk bottles, with an inoculation of fresh yeast, was used. As soon as the larvae reached full size and began to leave the medium, a layer of sawdust was added. This prevented the adults from obtaining any food until they were transferred to test bottles. The flies were used as soon as possible, never more than 24 hours after emergence.

Test bottles were set up as follows: solutions to be tested were put into 10 ml. vials stoppered with a roll of filter paper which served as a wick. About 50 ml. of 1.5 per cent agar was poured into a half-pint milk bottle: this maintained moisture and facilitated counting dead flies. For non-fermentable substances the vials were simply embedded in the agar base, otherwise they were wrapped in strips of paper toweling to form a plug for the milk bottle. This stopper could be changed readily and fresh solutions offered the flies, eliminating the complications of bacterial growth. It was found desirable to transfer the flies to fresh bottles after about two weeks if they survived, since otherwise dead flies were eaten by larvae and counting became difficult.

One hundred flies were used for each test. They were divided among three bottles for convenience in counting. The dead flies in the bottles were counted each day. Initially the number of days required for 50 per cent of the flies to die was used as a means of evaluating the degree of utilization of a substance, but it was found that many of the materials having low values could not be differentiated without making counts at shorter intervals, which was impractical. A better index was achieved by totalling the daily survival percentages and using the resulting number as an index of nutritive value. For example, when formic acid was fed to flies, all survived the first day, 43 per cent the second, none the third. The "score" was, therefore, 143.

Larvae. Three of the common sugars were tested on sterile larvae. Eggs were obtained by allowing flies to deposit them on small dishes of agar for about two hours; the eggs were then collected and sterilized by immersion in 85 per cent alcohol for 10 minutes and transferred to shell vials containing 10 ml. of sterile culture medium. Each vial contained the following: powdered agar, 150 mg.; dried brewer's yeast, 50 mg.; sugar, 50 mg.; distilled water, 10 ml. The same medium, minus sugar, is the "starvation diet" of Beadle et al. (1938), and this, together with their "adequate" diet of 2 per cent yeast, was used for comparison with the sugar supplemented media.

Each vial was seeded with 40 eggs and maintained at 25° C. After the formation of pupae, the vials were examined daily and when all the adults had emerged, counts were made to ascertain: (a) number of adults; (b) number of pupae not completing metamorphosis; (c) number of unhatched eggs. The larvae sometimes churned the medium so that unhatched eggs were lost, but a large number of vials were found with eggs and egg cases undisturbed; from these it was calculated that an average of 4 eggs per vial failed to hatch. The numbers of eggs given in Table IV represent, therefore, 36 eggs per vial.

Results

If flies are put into dry bottles, they are all dead within 48 hours: their score is 65. If a layer of agar is put into the bottles, the score is 110; if, in addition, a vial of distilled water is supplied, the score rises to 120. On standard corn meal, agar, and sugar medium, they live a long time: the score for that is 4418.

Table I shows the scores calculated as described above, and the day on which 50 per cent of the flies in each test were left alive. From the data it can be seen that adults of *Drosophila melanogaster* can live on a large number of substances in several classes of chemical compounds, but that the sugars and their close derivatives are best for maintaining these insects. Even in the sugars, each subgroup is found to contain substances which cannot be utilized.

If flies are supplied with pure sugar solutions, they survive for periods dependent upon the degree of utilization of the sugar and its concentration. Poorly utilized sugars like xylose sustain life only for short periods, even in concentrated solutions, while well utilized sugars like sucrose maintain life for longer and longer periods as the concentration increases. The limit in this direction seems to be reached between M/10 and M/5 for sucrose, for further increases in the concentration fail to increase survival. Groups of flies tested with concentrations of sucrose as follows: M/5, M/2, M, and 2M gave results no better than M/10, and indeed, the higher concentrations showed a tendency to decrease the life span slightly, but other factors such as osmotic pressure might enter to account for this.

The substances which were tested gave scores ranging from that of raffinose, 2600, to guanine, 13, as shown in Table I. Three groups of substances can be distinguished:

Group 1. Substances which appear to be inert, with scores close to that of water. Because of the natural variability of different batches of flies, and temperature conditions as noted previously, one could not expect sharply demarcated groups, and in fact there is a continuous gradation of scores. Probably all substances with scores between 100 and 150 should be called inert. This group would

TABLE I

The survival of adult Drosophila melanogaster on various substances, given as summations of daily survival percentages (A), and as days required for 50 per cent mortality (B). Except where noted, solutions are M/10. Each test represents 100 flies.

	A	B
Controls		
Dry bottle	65	1
Bottle with agar	110	2
Water (442 flies)	120	2
Standard medium	4418	45
Pentoses		
D-Xylose, M/2	680	7
Ribose	340	4
D-Xylose	211	3
L-Fucose	169	3
D-Arabinose	166	3
D-Xylose, M/20	131	2
L-Arabinose, M/2	101	2
L-Rhamnose, M	80	2
D-Arabinose, M/2	69	2
L-Rhamnose	68	2
L-Arabinose	64	2
Hexoses		
D-Fructose	1855	18
Glucose	1521	16
D-Mannose	1415	14
D-Fructose, M/20	1033	11
D-Galactose	945	9
Gluctose, M/20	663	7
D-Galactose, M/20	235	3
L-Sorbose	191	3
L-Sorbose, M/2	68	2
Disaccharides		
Sucrose	2218	24
Sucrose, M/5	2141	23
Maltose	2040	17
Sucrose, M	2010	22
Trehalose	1864	21
Maltose, M/20	1668	16
Sucrose, M/2	1624	20
Sucrose, 2M	1516	16
Sucrose, M/20	1506	14
Melibiose	1237	12
Sucrose, M/40	382	4
Lactose	179	3
Lactose, M/2	153	2
Lactose, M/20	100	2
Cellobiose	84	2
Cellobiose, M/2	40	1
Trisaccharides		
Raffinose	2600	28
Melezitose	2432	26
Raffinose, M/20	1460	15
Melezitose, M/20	909	14
Polysaccharides		
Dextrin, 1%	778	8
Starch, 1%	334	4
Glycogen, 1%	298	4
Inulin, sat. sol.	160	2
Alcohols		
Ethyl, M/5	172	3
Ethyl, M/2	99	2
Ethyl, M/10	93	2
n-Butyl	102	2
tert-Amyl	100	2
n-Amyl	99	2
iso-Butyl	96	2
sec-Butyl	95	2
tert-Butyl	50	
Polyhydric alcohols		
Glycerol	1369	14
Mannitol	729	6
Inositol	572	6
Sorbitol	358	5
Adonitol	308	4
m-Erythritol	170	3
Dulcitol, M/5	119	2
Dulcitol	108	2
Arabitol	107	2
m-Erythritol, M/2	86	2
penta-Erythritol, M/2	51	1
M/10	40	1
Glycols		
Propylene	172	3
Diethylene	160	2
Ethylene	124	2
Dipropylene	60	2
Carboxylic acids		
Butyric	205	3
Acetic	202	3
Formic	143	2
Valeric	133	2
Propionic	113	2
Lactic, M/2	377	5
M/5	327	4
M/10	208	3
M/20	153	2
Pyruvic, M/5	100	2
M/10	90	2
M/20	75	2
Glycolic	107	2
Levulinic	97	2
Succinic	367	4
Pimelic	160	3
Glutaric	124	2
Malonic	88	2
Azelaic	80	2
Adipic	70	2
Oxalic	20	1
Malic	234	3
Aconitic	162	3
Itaconic	158	3
Fumaric	151	2
Maleic	120	2
m-Tartaric	97	2
Citric	413	4
Salts		
Sodium succinate	115	2
Sodium citrate	105	2
Sodium lactate	115	2
Sodium malonate	99	2

TABLE I—*Continued*

	A	B		A	B
Amino acids			Miscellaneous		
Glycine	202	3	Yeast-sucrose, equal parts, dry	2074	24
DL-Methionine	195	3	alpha-Methylglucoside	639	6
L-Glutamic acid	124	2	Yeast, fresh 2% suspension	165	3
DL-Aspartic acid	122	2	Parenamine, 1% (proprietary casein hydrolysate)	147	2
DL-Alanine	108	2			
Beta alanine	108	2	Amygdalin	139	2
L-Cystine (sat. sol.)	102	2	Yeast, fresh dry	128	2
L-Cysteine	101	2	Catechol	126	2
DL-Glutamic acid	101	2	Albumin, 1%	117	2
DL-Threonine	101	2	Lecithin, 1%	116	2
L-Arginine	95	2	Charcoal, dry	107	2
DL-Phenylalanine	93	2	Glucosamine	106	2
L-Histidine	89	2	Casein, dry	106	2
DL-Isoleucine	72	2	Gulonic lactone, 4%	105	2
L-Lysine	71	2	Magnesium hexosediphosphate	104	2
L-Proline	70	2	Glucoheptonic lactone, 4%	100	2
L-Leucine (sat. sol.)	67	2	D-Galacturonic acid	98	2
L-Hydroxyproline	66	2	Xylan (sat. sol.)	94	2
DL-Tryptophane (sat. sol.)	63	2	Sucrose acetate	93	2
L-Tryptophane (sat. sol.)	62	2	Mucic acid	90	2
L-Tryosine (sat. sol.)	57	2	Calcium glucoheptonate, 4%	84	2
DL-Leucine (sat. sol.)	55	2	Nucleic acid (sat. sol.)	83	2
DL-Norleucine	55	2	Sodium nucleate, 1%	82	2
DL-Serine	51	1	Yeast, dried, suspension	80	2
DL-Valine	51	1	Milk, powdered	78	2
			Yeast, dried	64	2
			Starch, Lintner, dry	45	1
			Xanthine (sat. sol.)	15	1
			Guanine	13	1
			Uracil	13	1

include not only substances not utilized when ingested, but those which might be utilized somewhat, were they not also slightly repellent so that the flies do not drink the solutions.

Group 2. Substances which are utilized by Drosophila, shown by scores higher than that of water. This group includes anything which prolonged the life of the flies in any degree, from such poor nutrients as xylose to the best of the higher sugars. Sugars, particularly the mono-, di-, and trisaccharides, lead in this group, but moderately good results were obtained with dextrin, glycerol, mannitol, inositol, and alpha-methylglucoside. Some prolongation of life was obtained with starch, glycogen, sorbitol, adonitol, and with butyric, acetic, lactic, succinic, malic, and citric acids. The only amino acids showing any usefulness were methionine and glycine. A few other substances, such as ethyl alcohol, propylene and diethylene glycol, aconitic and itaconic acids, were doubtful. Proteins alone, e.g. albumin, were of no value, nor were such products as casein, yeast, or milk. The low values obtained with dry yeast (64) and starch (45) prompted a test with an inert powder. Charcoal was selected, and the relatively high score (107) suggests that there is something definitely harmful in dry starch and yeast, but whether its nature is

physical or chemical has not yet been ascertained. Dry yeast mixed with an equal amount of powdered sugar, on the other hand, makes an excellent food, giving a score of 2074.

In order to obtain a more exact comparison of nutritive value among some of the commoner sugars, seven were tested under identical conditions. The molarities of the solutions were chosen to equate the mono- and disaccharides with respect to weight per unit volume. Lactose, M/20, and xylose, M/10, showed no nutritive value, and galactose, M/10, very little. The other sugars were, in order of increasing nutritive value: glucose, M/10, 1375; sucrose, M/20, 1440; maltose, M/20, 1720; fructose, M/10, 1833. These scores and the curves of Figure 1 show there was little variation in this group, also that the results were nearly the same as those shown in Table I for the larger series of experiments.

The longevity of flies fed on di- and trisaccharides was compared, under identical conditions, with that of flies fed on the constituent monosaccharides. Table II

TABLE II

A comparison of some di- and trisaccharides with their hexose constituents. Each pair was run with flies from the same batch, under identical temperature conditions.

Substance	Conc.	Score	Substance	Conc.	Score
Sucrose	M/20	1455	Raffinose	M/20	1460
Fructose Glucose	M/20 M/20	1421	Fructose Glucose Galactose	M/20 M/20 M/20	1492
Maltose	M/20	1466			
Glucose	M/10	1363	Melezitose	M/20	1257
Trehalose	M/20	1064	Glucose Fructose	M/10 M/20	1285
Glucose	M/10	1285			

shows that there was little difference in the results, a mixture of fructose and glucose being as good as an equivalent amount of sucrose, etc.

Larvae. The results obtained in rearing sterile larvae on yeast and on yeast-sugar mixtures are given in Table IV. No significant difference was found in the number of flies produced by the three sugar media. A significant difference was found when adequate amounts of yeast were supplied, and an increase in the amount of sugar might have increased the yield. Since the object of the experiment was to differentiate among the sugars, if possible, by putting the larvae into somewhat unfavorable conditions, this was not done. Flies consuming fructose developed more rapidly than those on sucrose and glucose, though less rapidly than those having a full yeast diet.

Group 3. Substances which have low scores, and are therefore toxic or repellent. Flies in a bottle having a layer of agar live almost as long as if they are supplied with drinking water. Substances which are merely repellent will, therefore, be difficult to separate from those which are nutritionally inert. Toxic sub-

stances should give much lower scores and be accordingly easier to single out. Guanine, for example, is clearly toxic. Variations in toxicity and in the flies themselves naturally militate against any sharp distinction, so that further experiments were performed to bring out hidden differences. The difference between toxic and repellent substances can sometimes be demonstrated readily by offering a questionable solution alone and in combination with a separate vial of water. Rhamnose alone, for example, gave a score of 68, but when the flies were offered

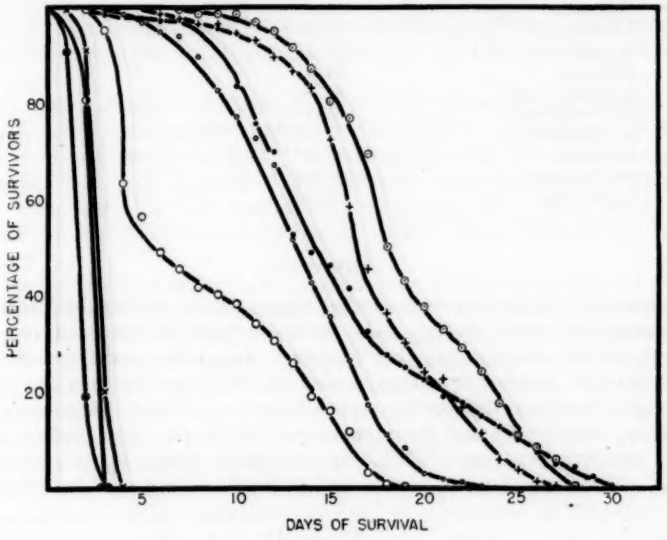

FIGURE 1. The duration of life of adult fruit flies fed solutions of various sugars. Lactose, M/20, ⊗; water, ◑; xylose, M/10, ×; galactose, M/10, ○; glucose, M/10, ○; sucrose, M/20, ●; maltose, M/20, +; fructose, M/10, ⊙.

an additional vial of water, the score rose to 100. No discrimination was evidenced, and presumably the flies lived longer because they drank less of the rhamnose solution. When repellency is suspected, however, something must be used to insure the ingestion of the solution. Vogel (1931) used sucrose solution, and a M/40 solution of sucrose was found useful in these experiments. Testing a large number of flies with this solution alone gave a score of 382. Table III shows how the results differed when various substances were added to it. Dulcitol alone is seemingly inert in M/10 solution, but when M/40 sucrose is added, the flies live longer than in sugar alone (score 508). Isoleucine is inert either way. D-Arabinose, on the other hand, prolongs life slightly when alone but shortens it when added to the sucrose solution, a puzzling result, to be sure. Sorbose would seem to be toxic either alone or in sucrose solutions, as do tartaric acid, norleucine and histidine, while valine, which is toxic when alone, can probably be detoxified when sucrose is present.

TABLE III

The effect of certain substances on Drosophila when dissolved in water and in M/40 sucrose. Each pair run under identical conditions.

Substance	Conc.	Score	
		In water	In M/40 sucrose
Cellobiose	M/10	84	396
Dulcitol	M/5	119	508
D-Arabinose	M/5	170	162
L-Sorbose	M/2	68	285
M-Tartaric acid	M/5	102	124
D-Tartaric acid	M/5	80	115
DL-Norleucine	M/10	20	83
DL-Valine	M/10	24	353
DL-Isoleucine	M/10	111	360
L-Histidine	M/10	93	203

DISCUSSION

As noted above, the question of what sugars can be utilized by insects has been investigated for several species. The results in hand for the adult and larval bee, the adult blowfly, and for the adult fruit flies *Anastrepha* and *Drosophila,* indicate almost identical abilities to utilize sugars, as nearly as the data are comparable. The really clear cut differences reported are as follows: mannose is used by *Calliphora, Anastrepha* and *Drosophila,* but not by the bee. Indeed von Frisch (1934) and Staudenmayer (1939) have reported a specific toxicity of mannose for the bee. Melibiose, dextrin, starch, and glycerol are not used by adult bees, but

TABLE IV

The development of sterile Drosophila larvae on low yeast, low yeast plus sugars, and adequate yeast diets.

Medium	Number of eggs (36/vial)	Number of pupae	Mean number of pupae per vial	Difference divided by prob. error of difference	Number of adults	Mean number of adults per vial	Difference divided by prob. error of difference	Mean number of days for emergence of all flies
0.5% yeast	252	73	10.3±2.9*	2.0	67	9.6±3.3*	2.0	20.5±3.4*
0.5% yeast 0.5% glucose	180	106	21.2±4.1	0.8	102	20.4±4.3	0.6	21.0±1.7
0.5% yeast 0.5% sucrose	540	371	28.5±2.3	2.0	353	27.1±2.3	1.4	21.5±1.3
0.5% yeast 0.5% fructose	216	183	30.5±1.0	3.0	178	29.7±1.8	4.5	16.2±3.7
2.0% yeast	72	70	35.0±0.9		70	35.0±0.9		12.0±0.0

* Probable error.

are by *Calliphora* and *Drosophila*. Inositol is utilized by *Drosophila*, but not by the others, and arabinose is used by *Apis* alone. There are other differences reported, such as the use of fucose by *Drosophila* and not by other forms, but the degree of utilization is so small that the difference is unimportant. The present experiments do show, however, that no substance should be judged inert until it has been tested in several concentrations, e.g. xylose is very poor in M/10 or less, but definitely useful in M/2. Also, substances should not be finally classified as useless or toxic unless they are offered in such form that ingestion is certain. Dulcitol, for example, is apparently inert for *Drosophila* when given alone, even up to M/5, yet when it is dissolved in M/40 sucrose, the flies live longer. The comments of Vogel (1931), Haslinger (1935) and Fraenkel (1940) are also pertinent to this point.

The ability of *Calliphora* and *Drosophila* to utilize glycogen and starch is clear, although it is much less than the ability to utilize sugars. The danger of using a partially hydrolyzed starch should be noted. *Drosophila* fed Lintner's soluble starch, one per cent, gave a score of 625, whereas sugar-free corn starch scored only 334. Reducing sugar was readily demonstrated in the soluble starch, which may account for the partial development of *Aedes* larvae reported by Hinman (1933).

The question of which sugar is best, which was raised by Bertholf (1927), is, perhaps, one applicable only to the individual species. It is further complicated by the variety of standards adopted by various investigators. Yet it is interesting to note that the "physiological sugar," glucose, is consistently poorer than others, being rated second by Phillips, third by Baker and Fraenkel, and fourth by Bertholf and in the present experiments, when only sucrose, maltose, glucose, and fructose are considered. Fructose, on the other hand, is rated first by Phillips, equal to sucrose by Fraenkel, second to sucrose by Bertholf, and in the present experiments it was superior to the others. Indeed, a comparison of scores for M/10 fructose and M/20 raffinose indicates that fructose is superior to the trisaccharides also. Sucrose is at or near the top in all.

The curve for galactose in Figure 1 is also of some interest. The initial mortality was so heavy that it suggested reduced powers for utilization of galactose, or greater power of mobilizing enzymes, on the part of one of the two portions of the population. A repetition of the experiment yielded similar results. The basis of the variability is not known but it will be investigated.

Partial successes were obtained with the substances regarded as intermediate products of carbohydrate metabolism. None of these was utilized by *Calliphora* (Fraenkel); *Drosophila*, however, survives a short time on citric, malic, succinic, lactic, butyric, and acetic acids, and possibly also on aconitic, itaconic, fumaric, and pimelic acids, although these are on the borderline. Since there is such close agreement in other respects, these data suggest that the blowfly might be able to metabolize the compounds in question, a possibility which Fraenkel has pointed out. In an experiment in which the present technique was used with *Lucilia sericata*, the flies died about as rapidly when offered M/10 citric acid or dry citric acid as they did when offered water alone. *Calliphora* was not available for this test, but the results with *Lucilia* suggest that if blowflies are able to metabolize any of the intermediates, some other means must be employed for introduction of the material.

According to Weidenhagen (1931), and the somewhat modified point of view

of Pigman (1944), all carbohydrates can be split by a small number of enzymes. With Weidenhagen's work in mind, Fraenkel concludes that only two enzymes, an alpha-glucosidase and an alpha-galactosidase, need exist in *Calliphora* to split all the carbohydrates that the blowfly utilizes. *Drosophila* evidently depends largely on the same two, but may have in addition a fructofuranosidase, which would be needed to utilize inulin, and could also act on sucrose. An amylase, too, must be present to split starch and glycogen.

While the longevity of the fruit fly on sugar alone may seem remarkable (50 per cent survival up to four weeks), the much greater longevity on the standard culture medium which furnishes carbohydrate directly and protein and accessory factors from the yeasts growing on the medium suggests that the addition of traces of other substances to the sugar solution might increase survival greatly. A further point on longevity is that the present method is not calculated to produce the longest lived flies. According to Pearl, Miner and Parker (1927), the maximum longevity of *Drosophila* is found in relatively crowded populations, about 50 flies in a 30 ml. vial having given best results in their experiments.

Summary

1. *Drosophila melanogaster* can survive for varying periods on pure solutions of many compounds, including sugars, polysaccharides, polyhydric alcohols, aliphatic acids, etc.

2. In equivalent solutions, the order of usefulness of some common sugars was found to be: fructose > maltose > sucrose > glucose > galactose > xylose > lactose.

3. There is no significant difference in life span between flies fed on disaccharides and their constituent monosaccharides.

4. Doubtful sugars can usually be resolved into toxic, repellent, or slightly useful substances by offering them in dilute sucrose solutions.

5. On a sterile, "starvation" diet, larvae develop better on fructose than on glucose.

6. On the basis of survival when fed pure substances, *Drosophila* seems to possess alpha-glucosidase, alpha-galactosidase, beta-fructofuranosidase and amylase.

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RESPIRATION OF OOCYTES, UNFERTILIZED EGGS AND FERTILIZED EGGS FROM PSAMMECHINUS AND ASTERIAS

HANS BOREI

Wenner-Gren's Institute for Experimental Biology, University of Stockholm

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1. Introduction

Using Cartesian diver micro-respiration technique Lindahl and Holter (1941) measured the oxygen consumption rate of primary oocytes, mature unfertilized eggs and fertilized eggs of the sea-urchin *Paracentrotus lividus*. They found that the oocyte respiration is markedly higher than that of the unfertilized egg and that it probably exceeds that of the newly fertilized egg. On the other hand Boell, Chambers, Glancy and Stern (1940) stated, in an earlier brief note, that in similar diver measurements the mature unfertilized *Arbacia* egg reveals a higher oxygen consumption and a higher capacity to oxidize p-phenylenediamine than does the immature egg (oocyte).

Because of these two quite divergent results it was considered to be of interest to investigate, and eventually settle the matter, by using a third sea-urchin species. *Psammechinus miliaris* from the Swedish West Coast was chosen. This species offers some special advantages: its spawning period is relatively long; its oocytes can be obtained regularly during practically the whole of the spawning period; and, there are three distinct cytoplasmic maturity stages of the egg, viz., under-ripeness, ripeness and over-ripeness. These maturity stages are characterized by differences in fertilizability, in fertilization membrane appearance and in reaction to hypertonic medium (cf. Runnström and Monné, 1945). Thus it might be possible to follow in detail any gradual alteration in oxygen consumption during the entire egg maturation process. Since it is necessary selectively to pick out the desired cells from the cell mixture, which is extruded from the ovary, the diver technique, which permits the measurement of the oxygen consumption of as few as about a hundred oocytes or resting eggs, will be very suitable. As only a small number of cells are

necessary for each experiment, it is possible to charge any desired parallel diver unit with cells from the same ovary.

Furthermore, it was thought that a comparison between the sea-urchin oocyte or egg respiration and that of the starfish oocyte or egg might throw some light on the general laws of echinoderm egg metabolism. Tang (1931), using a Warburg technique, found that mature and immature (oocytes) *Asterias* eggs used up the same oxygen amount per time unit. Boell, Chambers, Glancy and Stern (1940) arrived at the same result using diver technique, but Brooks (1943) in Warburg experiments found a lower consumption in immature eggs than in mature. In the present paper diver measurements on *Asterias glacialis* oocytes and eggs will be reported.

In addition, diver measurement data concerning the oxygen consumption of the fertilized sea-urchin and starfish egg are given and interpreted. In *Asterias* the respiration is only followed over the first mitosis, but in *Psammechinus* it is measured until some hours after hatching.

Abbreviations used in the text:
Ps.: Psammechinus miliaris
Ast.: Asterias glacialis
Par.: Paracentrotus lividus

2. General Remarks on Material and Methods

2.1. *Animals*

Ps. occurs in two phenotypic varieties: one, called the *Z-form*, is the trivial littoral form; the other, called the *S-form*, is found at greater depths. They differ in size and morphological appearance and, of special interest in this investigation, in the spawning period. The *Z-form* has fertilizable eggs from the middle of June until the middle of July, the *S-form* during July and August. Concerning living conditions, distribution and biology, cf. Lindahl and Runnström (1929) and Borei and Wernstedt (1935).

The *Ps. Z-form* animals were dredged from about 6 m. depth. They were kept in a wire mesh cage immersed in the surface water off the station pier, where temperature and salinity conditions were approximately the same as those at the dredging-locality. The sea-urchins were used for experiments within a few days of being caught.

The *Ps. S-form* sea-urchins were caught at about 20–30 m. depth. They were brought to the station immersed in 32–33 ‰ salinity water in a big Dewar vessel to keep the temperature low. At the station they were transferred to aquaria with running sea water, where the salinity was about 32–33 ‰ and the temperature between 15–17° C. The conditions at the dredging-localities were about the same with regard to salinity, but somewhat lower as regards temperature. The animals were mostly used for experiments on the day of capture; in some rare cases they were not used until the next day.

Ast. was dredged from 30–40 m. depth. The animals were brought to the station and kept there in the manner described above for the *Ps. S-form*. They were invariably used for experiments on the day of capture. Their spawning period falls mainly in May and June.

2.2. *Diver technique*

The technique of Cartesian diver measurements has been described in detail by Holter (1943). Only points of special interest will be mentioned here.

The divers used were of standard type (volume 8–10 µl.) made of Jena Geräte glass ($\phi = 2.412$). They were charged as follows, according to the "Diver charge Type I" of Borei (1948):

Mouth seal: Holter's medium ($\phi_M = 1.325$)
Neck seal I: 0.5 µl. paraffin oil ($\phi_{OI} = 0.87$)
Neck seal II: 0.5 µl. isotonic sodium hydroxide
Bottom drop: 0.8 µl. sea water cell suspension
($\phi_W \leq 1.0$)
(cf. Borei (1948), Figure 1: I).

The cells were extruded from the ovary after this had been removed from the body, sifted through bolting-cloth and washed three times in sea water before being picked up in a braking pipette.

The salinity of the sea water of the cell suspension varied according to the material. For the *Ps. Z-form* water of 24.6 ‰ S was mostly used. This salinity figure approximately equals the medium salinity during the summer months of the surface water off the station pier where the animals were kept. Occasionally higher salinities occur. Thus it was sometimes found more correct to apply water of 27 or 29 ‰ S. For the *Ps. S-form* which lives in water of higher salinity and was therefore kept in the station aquarium sea water; 32–33 ‰ S sea water was used. In the *Ast.* experiments the salinity was 29 ‰ throughout. This salinity is somewhat lower than that on the dredging-localities, but had to be used owing to some temporary trouble with the station sea water pipe-line. All salinities were checked by titrimetric estimations according to Borei (1947). For pH control potentiometric measurements (glass electrode) were employed. Isotonic NaOH solutions for the diver neck seal II were prepared from a stock solution. (From the sea water freezing points tabulated by Knudsen (1903) it can be calculated that 0.365 *N* NaOH is isotonic to 25 ‰ S sea water.)

The temperature in the experiments was mainly 18° C. For some *Ps.* experiments temperatures between 15–21° C. were employed, owing to the requirements of simultaneous measurements for other investigations. The maximum temperature for normal larval development of *Ps.* was studied by Runnström (1927) and found to be 22° C. *Ast.* belongs to the same species group, the mediterranean-boreal, and is likely to have about the same upper temperature limit for normal development. The temperatures in the experiments are thus well below the critical level.

The number of eggs per diver was 40–50 for *Ast.*, 70–120 for *Ps.* when unfertilized, and 40–50 for *Ps.* when fertilized. These numbers give approximately 12.5, 8 and 9×10^{-3} µl. oxygen consumed per hour respectively in the most crowded divers, *i.e.* δp lies between 1–2 cm. per hour. This rate is best suited to keep the errors of the diver apparatus low (cf. Holter, 1943) and lies, moreover, within the range $3\text{–}18 \times 10^{-3}$ µl. per hour, which Lindahl and Holter (1940) in diver experiments on *Par.* found to be characterized by direct proportionality between number of cells and oxygen consumption. Lindahl and Holter (1940) further found that diver and Warburg experiments which were performed simultaneously gave very consistent results. The same applies to the present diver experiments on unfertilized *Ps.* eggs compared with the Warburg experiments of Borei (1934) (cf. 3.112.2). Thus the oxygen supply is apparently not the limiting factor in these diver experiments. This view is further supported by the fact that in the course of the experiments the oxygen pressure within the diver does not decrease more than 2 mm. Hg at the most, whereas it is generally agreed (cf. Tang, 1941) that the sea-urchin egg respiration is unaffected by a decrease in oxygen pressure from 160 mm. down to 40 mm. The number of cells per volume of cell suspension is about the same in diver experiments as in Warburg ones, or slightly lower.

After completed diver measurements the cells were washed out of the divers with sea water, re-counted and then microscopically observed as to condition and development. In applicable cases even fertilizability controls were undertaken. Only those experiments were accepted in which the cells passed these post-diver-measurement controls.

2.3. *Evaluation of results*

The oxygen used up during the experiment, δv, is calculated from the read pressure difference, δp, according to the formula

$$\delta v = \frac{V \cdot \delta p \cdot T_0}{p_0 \cdot T}. \qquad (1)$$

Using this formula δv will be given in μl. measured at 0° C. and normal barometric pressure, provided δp is stated in cm. Brodie, p_0 is the normal barometric pressure in cm. Brodie (= 1000), T is the temperature of the experiment in °K., T_0 stands for 273° K., and if V stands for the total gas space in μl. of the charged diver at equilibrium pressure. It will be noted that this formula is similar to that used by Holter (1943) p. 466 in cases where the solubility of the measured gas is low, and where formed CO_2 is absorbed away, but a temperature correction has been added in order to render a comparison between the measurements possible even if the latter have been taken at different temperatures (see, however, below). In the formula the absorption of oxygen in the liquid phases of the charge has been disregarded as it is a very small quantity.

From the diver equation given by Linderström-Lang (1943) p. 363 the following expression for V may be derived:

$$V = g_D \left[\frac{1}{\phi_M} - \frac{1}{\phi_{Gl}}\right] + V_W \left[\frac{\phi_W}{\phi_M} - 1\right] + V_{Oi} \left[\frac{\phi_{Oi}}{\phi_M} - 1\right] \qquad (2)$$

where g_D = weight of the empty diver in mg.,
V_W = volume in μl. of aqueous charge (*i.e.* in the present case: cell suspension in bottom drop + hydroxide solution in neck seal II),
V_{Oi} = volume in μl. of paraffin oil in neck seal I,
ϕ_W, ϕ_{Oi}, ϕ_M and ϕ_{Gl} = densities of the aqueous charge, the paraffin oil, the medium and the diver glass.

The formula may be shortened to:

$$V = g_D \cdot A + B + C \qquad (3)$$

where $g_D \cdot A$ may be defined as the total gas space in μl. of the uncharged diver at equilibrium pressure. This is a constant characteristic of the individual diver. For calculating A the graph in Figure 1a may be of help. For a given medium (in this investigation Holter's medium, $\phi = 1.325$; cf. Holter (1943) p. 412, has been used) A is solely a function of ϕ_{Gl}.

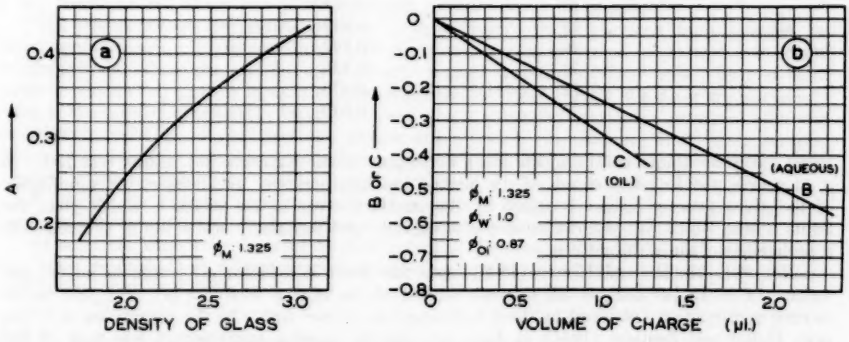

FIGURE 1. Graphs for calculating the values of the constants A, B and C in the formula for V, the total gas space of the charged diver at equilibrium pressure.

The constants B and C are governed by the adopted type of charge, but are independent of the characteristics of the diver. They may be found from the graphs in Figure 1b.

The value of A ought to be stated to the third figure; B and C will be sufficiently correct if stated to the second.

As stated in 2.2., measurements were usually performed at 18° C., but in some experiments on *Ps.* cells the temperature was varied within the limits 15–21° C. Measurements at different

temperatures are not comparable if merely calculated according to formula (1), as the cellular oxygen consumption rate is itself a function of temperature. As will be shown in a later paper (jointly with S. Lybing) this function in the unfertilized *Ps.* egg is characterized by a $Q_{10} \simeq 2.25$. Adopting this value, the consumption rates at any temperature within the range may be converted to consumption rates at 18° C. The same applies when the results of other investigators are compared with those given in this paper. The same Q_{10} value may, without significant errors, be used even for fertilized eggs.

If all corrections and constants are now put together the consumption formula (1) is simplified to:

$$\delta v = V \cdot \delta p \cdot K. \tag{4}$$

The value of K for different experimental temperatures when adopting $Q_{10} = 2.25$ will be found from Table I.

The technical errors of the method will no doubt remain below 5 per cent (cf. Holter, 1943). The biological scattering of the gained figures is, however, much greater. (This scattering is frequently met with by investigators who study oxygen consumption in marine invertebrates. Thus, using apparently identical objects one frequently comes across biological scattering amounting to the horrifying value of over 100 per cent.) In order to get a measure on the biological scattering in this investigation the standard deviation, σ, has been used. This

TABLE I

Value of K in the ultimate diver gas exchange formula if $Q_{10} = 2.25$

Temperature in experiment °C.	K
14.5	1.264×10^{-3}
15	1.210
16	1.111
17	1.020
18	*0.938*
19	0.862
20	0.792
21	0.728
22	0.670
23	0.618

figure gives the limits within which about two-thirds of the experimental results will fall. In σ, of course, the technical errors of the method are incorporated. In addition, the significance of the given average values is stated by the standard error of the mean, ϵ, which gives the limits within which the real center of the biological (and technical) scattering is situated with a probability of about 70 per cent.

For *Par.* Lindahl and Holter (1940) state that there is a biological scattering in the egg volume of 10–15 per cent, in the enzyme content of the egg of about 10 per cent, and in the oxygen consumption (obtained by diver technique) of 10 per cent. In the respiration of *Ciona* eggs Holter and Zeuthen (1944) in diver experiments found a scattering of less than 20 per cent, and in the egg volume less than 16 per cent. (Cf. Zeuthen (1947a), who on pp. 44–48 has discussed the influence of biological factors on metabolic measurements, especially on such as are performed with diver technique.)

In the present investigation the standard deviations for respiration measurements are found in Tables II, III, V and VIII, and for egg size in Tables IV and VI. To summarize it may be stated that for *Ps.* respiration there has been found a relative σ slightly greater than 20 per cent, and for *Ast.* a σ slightly greater than 15 per cent. The scattering in size is markedly smaller: for cell diameter $\sigma = 4.3$ per cent in *Ps.* and 3.5 per cent in *Ast.*, and for cell volume 13.5 per cent and 10.9 per cent, respectively.

3. Experiments and Interpretations

3.1. *Respiration of oocytes and unfertilized eggs*

3.11. *Psammechinus*

3.111. *Cell material*

In *Ps.* the egg may still be unfertilizable though full nuclear maturity has been reached, *i.e.* the polar bodies are expelled. This unfertilizability is due to the well known fact that in sea-urchin eggs nuclear maturity is not followed by cytoplasmic maturity until considerably later. Such cytoplasmically immature *Ps.* eggs are characterized in the hypertonicity test (cf. Runnström and Monné, 1945) by rapidly appearing wrinkles which smooth out very slowly. These eggs are termed in this paper *under-ripe eggs.* When both nucleic and cytoplasmic maturity is reached, and the eggs can accordingly be fertilized, the wrinkles appear in 2–6 minutes after the eggs have been placed in hypertonic medium (2 ml. sea water + 0.6 ml. 2.5 *N* NaCl) and smooth out in about 40 minutes. Such totally mature eggs are here termed *ripe eggs.* Later on the eggs again cease to be fertilizable. The wrinkles of the hypertonicity test are now poor or fail to appear and, if any do appear, they smooth out rapidly. These eggs are termed *over-ripe* (cf. also Wicklund, 1947).

These three classes of egg maturity are not mixed in one and the same female but in a catch of animals, females belonging to all these classes may simultaneously be found. In the beginning of the spawning season the females with under-ripe eggs prevail. At the end of the season not only females with over-ripe eggs become frequent, but there is also a marked rise in the number of females with under-ripe eggs (tendency to second spawning period?). This corresponds to the fact that the number of oocytes in the extruded egg-mass is higher both at the beginning and at the end of the spawning season than at its height.

The size of the oocytes varies greatly. Only such as were fully grown were accepted for diver measurements. To facilitate this such egg material was preferred as showed low oocyte percentage, *i.e.* where the oocytes were already mainly transformed into eggs. Such a state seems to ensure full growth in most of the remaining oocytes. During the diver measurements some of the oocytes occasionally started meioses which, when the eggs were examined after the experiment, could be judged from the broken down nuclear membrane. Such circumstances, however, could never be proved to effect perceptibly the oxygen consumption figures. The number, if any, of developing oocytes was always low (cf. also Lindahl and Holter, 1941).

From the *Ps.* material it was accordingly possible to pick out four different kinds of cells for diver experiments, viz. oocytes, under-ripe eggs, ripe eggs and over-ripe eggs.

In some few cases cells deprived of their jelly hull (treatment with acid) were used because they acted as controls in other experiments on naked cells. According to Borei (1948) there is, however, very little difference, if any, in oxygen consumption between coated and naked cells.

3.112. *Respiration of ripe eggs*

3.112.1. *Respiration curve*

In the ovary the germinal cells are continuously supplied with fresh metabolic material and may thus keep their respiratory rate at a constant level. When shed they are cut off from any additional supply and their metabolic rate is bound to drop gradually. This view is supported by the experimental results; see Figure 2.

The earliest respiration values after removal from the ovary cannot be stated with the diver technique. The time required for washing and picking out the cells,

for filling the diver, plus a minimum of time for attaining temperature equilibrium and then for obtaining two measurements suitably apart, cannot be pressed down to much under 40 minutes. The earlier values may, however, be found by extrapolation, but the most interesting period, viz. the shedding, will only be described rather approximately by this method.

A gradual decrease in the oxygen consumption of the shed unfertilized egg has already been reported for the *Ciona* egg by Holter and Zeuthen (1944) using Cartesian diver technique. Lindahl and Holter (1941) in their diver experiments on *Par.* have not stated the time after removal from the ovary, but point out that it would probably have been of importance. In fact, the results presented in Figure 2 show how necessary such a precaution is if comparable values are desired.

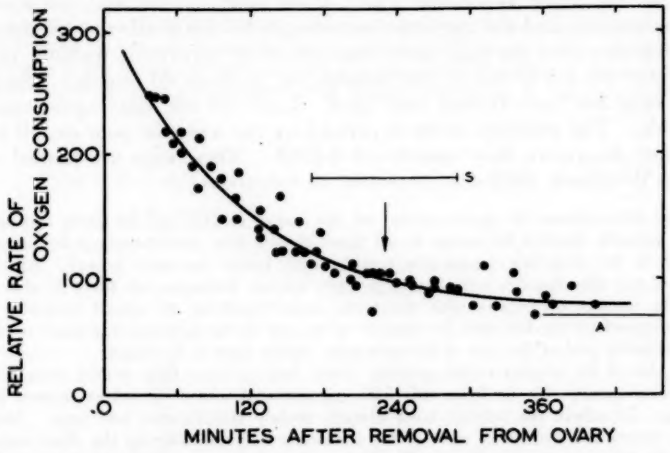

FIGURE 2. Relative rate of oxygen consumption of ripe *Psammechinus miliaris* eggs in relation to time after removal from ovary.

Results from 4 diver experiments. Values at 230 minutes (cf. 3.112.2) put = 100. Final asymptote (A) indicated. Arrow stands for chosen point of comparison for absolute rate measurements. Evaluated time span (S) in such experiments indicated.

The curve in Figure 2 closely resembles that of the endogeneous respiration of baker's yeast (Borei, 1942) where one is also concerned with cells cut off from external supply of metabolic material; the gradually decreasing respiration is composed of two parts. One proceeds monomolecularly and is thought to be governed by the rapidly decreasing amount of an initially present substrate. The other continues with a constant rate for a considerable time; its rate is thought to be limited by the available amount of an enzyme.

Mathematical analysis of the curve in Figure 2 shows that even the respiration of the unfertilized egg can be described in this way: There is a true monomolecular part of the respiration that rapidly tends to become zero, *i.e.* the curve approaches an asymptote (indicated in the figure). The amount below the asymptote represents the constant respiration part. This may probably be interpreted, as in the

case of yeast, as a simultaneous degradation of two different sources of metabolites over partly different enzyme systems.

3.112.2. *Absolute respiration values*

Because of the rapidly decreasing rate of oxygen consumption, as manifested in Figure 2, it is not convenient to compare values which are obtained from different materials during the first hours after the egg-removal from the ovary. Even small time differences may here cause considerable discrepancy in the consumption figure. Later on the curve becomes flatter and suitable for comparison with other experiments. The best thing to do would be to take the asymptote value, but this is impracticable by reason of experiment duration. Instead some point must be chosen on the part of the curve that has become reasonably horizontal, but which is still as close as possible to the point of egg-removal. Judging from the obtained curve, a point of time 4 hours from this event was chosen. In actual experiments 230 minutes from egg-removal was the average time, around which the measurements grouped themselves. Experimental conditions, however, have caused the center of the measurements in certain experiments to shift some 20 minutes in either direction. A total measurement-time of 2 hours, during which some six

TABLE II

Average rate (r) of oxygen consumption of ripe eggs of the two Psammechinus phenotypes 230 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Phenotype	r (μl./egg/hour)	σ	ϵ	n	Difference in r (μl./egg/hour)
Z-form	0.47×10^{-4}	0.12	0.02	25	0.06×10^{-4}
S-form	0.53×10^{-4}	0.17	0.03	39	($\epsilon_{diff.} = 0.03$)

equilibrium readings could be obtained, was thought fit for the evaluation of the consumption figure. The average position on the curve of the final oxygen consumption figure and the time span of the measurements used for its evaluation are marked in Figure 2.

The results from measurements in this manner on ripe eggs of both the *Ps.* phenotypes are recorded in Table II.

The figures for the two phenotypes differ slightly. The difference is, however, not greater than twice its standard error. Thus it has not been possible to establish any significant difference in oxygen consumption rate between the ripe eggs of the two forms (concerning egg volume, cf. 3.114).

Borei (1934) studied the oxygen consumption of ripe *Ps.* eggs by means of Warburg technique (20° C.) and found, in accordance with the above results, the figure 0.52×10^{-4} μl. per egg and hour (converted to be comparable to the figures in Table II, whereby the egg volume value from Table IV has been used).

Using Barcroft differential manometers (14.5° C.) Shearer (1922b) got a considerably lower value for the ripe egg, viz. 0.20×10^{-4} μl./egg and hour (converted

to be comparable to the figures in the present investigation). This may be due to imperfect measuring technique (too tightly packed eggs).

3.113. *Respiration of oocytes, under-ripe eggs and over-ripe eggs*

To get oxygen consumption values comparable to those of the ripe eggs precautions were taken, similar to those mentioned above, concerning the measurements. It was found that even the oocytes showed a decreasing respiration similar to that of the ripe eggs. A special stickiness of the oocytes, as reported for *Par.* by Lindahl and Holter (1941), was not found in the *Ps.* material.

The oxygen consumption figures obtained for oocytes, under-ripe eggs and over-ripe eggs are given in Table III, where, for comparison, the average value for the ripe eggs (calculated from Table II) is also included. The number of experiments on the different cell types is, however, too small to permit of any comparison between the two *Ps.* phenotypes, as was possible in the case of the more abundant material on ripe eggs.

TABLE III

Average rate ($\bar{r}$) of oxygen consumption of oocytes, under-ripe eggs and over-ripe eggs of Psammechinus 230 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Cell type	$\bar{r}$ (µl./cell/hour)	σ	ϵ	n
Oocytes	0.66×10^{-4}	0.13	0.04	11
Under-ripe eggs	0.56×10^{-4}	0.17	0.06	10
Ripe eggs	0.51×10^{-4}	0.16	0.02	64
Over-ripe eggs	0.45×10^{-4}	0.09	0.04	7

Obviously there is a gradual, though slight, decrease in oxygen consumption on advancing cell maturation (all differences are statistically *well* established except that between under-ripe and ripe eggs, which is probable but not fully secured).

Although the findings on the *Ps.* material are thus not fully in accordance with those of Lindahl and Holter (1941) on *Par.* they, nevertheless, principally show a lower respiration in the ripe egg in comparison with that in the oocyte. This question will be discussed more extensively later on (see 4). On the other hand, the statement of Boell and co-workers (1940) that the ripe eggs of *Arbacia* consume more oxygen than the immature eggs (oocytes) contradicts these findings. (Possibly the oocytes used in their experiments had been kept for an especially long time in sea water before diver measurements began.)

The relatively low *Ps.* oocyte respiration in comparison with that of the *Par.* oocyte may possibly be connected with some sort of species-specific metabolic change caused by the contact with sea water when the cell is shed. In order to estimate the oocyte respiration inside the ovary an attempt was made to obtain cells immersed in ovarial fluid by means of introducing the tip of a braking micropipette through the ovary wall into the cavity. The habitus of the ovary, which made it very difficult to remove the coelomic fluid from the surface of the ovary, together with its great fragility, made it extremely difficult to draw out pure ovarial

content. Two additional factors invalidated the reliability of the diver measurements, viz. first, the dense packing of the cells inside the ovary, which tended to overcrowd the diver bottom drop thus giving bad gas exchange conditions and also causing great difficulties when the oocytes were to be picked out from the extracted material, and second, the frequent occurrence of other coelomic cells, such as amoebocytes, etc., which invariably and inseparably accompanied the desired cells. (Cf. similar experiments on *Ast.* oocytes in 3.122, where all these difficulties were mainly absent.) In any case the diver measurements indicated that the initial oocyte respiration in ovarial fluid is of the same order of magnitude as the initial one in sea water.

3.114. *Size of cells*

The two *Ps.* phenotypic varieties, the *Z-* and *S-forms,* were previously (3.112.2) compared as to the oxygen consumption of the ripe eggs. No significant difference could be established. An attempt was then made to correlate the great biological variability of the oxygen consumption to the size of the eggs. No correlation could, however, be established between these two entities. If the amount of protoplasmic material has any influence on the rate of oxygen consumption, as might have *a priori* been expected, this influence is obscured by other experimentally uncontrolled factors governing the metabolic rate and the size.

TABLE IV

Diameter (d) and volume (v) of ripe eggs from the two Psammechinus phenotypes
n = number of females examined. At least 20 eggs from every female were measured

Phenotype	d (μ)	σ	ε	n	Difference in d (μ)	v (μl.)
Z-form	94.6	3.5	1.1	11	9.1 ($\varepsilon_{diff.} = 1.3$)	4.43×10^{-4}
S-form	103.7	5.1	0.7	49		5.84×10^{-4}

On the other hand distinct differences in size between the ripe eggs of the two forms definitely exist, as can be seen from Table IV. The significance of the difference is established by the fact that it is 7 times as big as its standard error. Similar results have been gained by Lindahl and Runnström (1929) (*Z*-eggs $d = 98.3\,\mu$; *S*-eggs $d = 114.8\,\mu$).

The size of the eggs is not a given constant. Thus, for instance, the *Z-form* eggs were found to be smaller in 1946 than in 1947 ($d = 87.5\,\mu$ and $d = 94.6\,\mu$, respectively) and Lindahl and Runnström (1929) state $d = 98.3\,\mu$ for eggs of animals from the same district in the late Twenties. Moreover, geographical dissimilarities also seem to exist: Thus Laser and Rothschild (1939) give $d = 103.3\,\mu$ for a Millport material and Hobson (1932) gives a volume corresponding to $d = 118.4\,\mu$ for a Plymouth material. These two materials are no doubt of *Z-form,* for which the figures $d = 87.5$–$98.3\,\mu$ were found at the Swedish West Coast (see above). Hydrographically caused nutritional differences are probably the chief reason for these dissimilarities.

Because of the simultaneous existence of full size oocytes and a variety of small ones, which are not fully mature, it is rather difficult to get a reliable figure for the

oocyte diameter. However, the oocytes seem, on an average, to be smaller than the eggs. This is supported by all measurements on material with apparently homogeneous oocytes, especially in the cases in which the oocyte percentage is low. For example a material with an average egg diameter of 96.8 μ showed an average oocyte diameter of 88.8 μ. These findings are in concordance with the results on *Ast.* (see 3.123), where the oocytes are quite homogeneous in size and where the eggs are bigger than the oocytes. Similar results are reported by Lindahl and Holter (1941) in *Par.*

It may be noted, first, that in *Ast.* the (primary) oocytes respire at a much lower rate than the eggs (or secondary oocytes), second, that in *Ps.* the oocyte respiration is instead slightly higher than the egg respiration, and third, according to Lindahl and Holter (1941), that in *Par.* the oocytes respire at a much higher rate than do the eggs. Thus the respiration cannot be correlated to the cell size and furthermore, as the amount of protoplasm will always be nearly the same in oocytes and eggs (though the degree of hydration may differ), it naturally cannot be correlated to the content of organic matter.

3.12. *Asterias*

3.121. *Cell material*

For *Asterias forbesii* Costello (1935) states that the nuclear membrane of the oocytes begins to disappear after about 10 minutes in sea water (19–26° C.) and that the first polar body is separated in about 60 minutes. After another 20 minutes the second polar body appears. Cytoplasmic maturity seems to be attained rather rapidly and to be connected with the diffusion of the nuclear sap into the cytoplasm. Chambers and Chambers (1940) confirm the maturation time schedule.

This scheme obviously also holds for the *Ast.* material of this investigation. When taken from the ovary the cells are, without exception, oocytes. (Recent authors agree that on natural spawning the cells are in a state of broken down germinal vesicle, *i.e.* primary oocytes with initiated first meiosis; cf. Hörstadius, 1939, and Runnström, 1944.) Within 30–40 minutes after being placed in sea water (18° C.) they usually show broken down nuclear membranes in over 90 per cent of the cells. At 60 minutes the first polar body can usually be seen and at 120 minutes often two (or three) are formed. The polar bodies are frequently dissimilar in size.

The natural stimulus for the oocyte development is the sea water (cf., *e.g.*, Hörstadius, 1939). This is clearly shown by the experiments with oocytes in ovarial fluid (3.122), where the meiosis was delayed for several hours. Small contaminations with sea water (or coelomic fluid), however, caused the seminal vesicle to break down rapidly.

After a few hours in sea water the cell material mainly consisted of eggs and a certain small percentage of primary oocytes. In addition, there were a number of secondary oocytes. These, however, were rather hard to distinguish from the eggs because they look exactly the same as the eggs. The only difference lies in the number of polar bodies, and this is extremely difficult to determine when picking out the cells with a braking pipette. Thus the material referred to as "eggs" in the diver experiments, may consist of a mixture of secondary oocytes and eggs. However, the respiration of these two kinds of cells seems to differ only very slightly, since the "egg" respiration agrees very well with that of the young fertilized egg (cf. 3.122). When taken out of the diver after the measurements and then more closely examined under the microscope most of the cells proved to be true eggs. Nevertheless the second meiosis might, of course, have taken place inside the diver during the oxygen consumption measurements.

Any noticeable break in respiration rate could, however, never be observed during diver measurements. Usually the "eggs" were picked out for the diver charge during the second hour in sea water.

The statement of Loeb and Wasteneys (1912) on unfertilized *Asterias forbesii* eggs, that these rapidly die if their oxidative processes are not suppressed, may also be true for the present material. In fact after some 24 hours a "dark" cytolysis is very common. For the diver measurements, which were mostly concluded within 4 hours after the removal from the ovary, this obstacle seemed to be without significance.

For the diver measurements on primary oocytes, such ones as have been mentioned before and which pass quickly over to eggs could not be used. Being a very homogeneous material they would have been very suitable, but measurements could hardly have started before they were already transformed. Thus for the diver experiments only such oocytes as remain as oocytes after some hours in sea water could be utilized. These cells have exactly the same appearance and size as the rapidly converting oocytes. The fact that they remain unconverted seems to point to some sort of immaturity. This might be the case, but only to a certain extent, for the majority of these eggs do actually convert later on, *i.e.* within some few hours after they have been taken out of the diver again for purposes of control. Thus one might be justified in thinking that the figures obtained on this material would not differ very much from those on rapidly converting oocytes, could they have been obtained. This view is supported by the fact that the obtained consumption figures are the same, irrespective of when the future conversion takes place and by the fact that it is possible to correct the obtained values for the oxygen consumption of such oocytes as have their germinal vesicle broken down when inside the diver (by applying the consumption value for eggs). Moreover, experiments with oocytes in ovarial fluid (in this case mostly "rapid" oocytes must be present) show consumption figures of the same order of magnitude as oocytes in sea water but not as eggs in sea water.

Only such oocyte experiments were accepted as, when examined immediately after the completion of the diver measurements, show no or very few oocytes with broken down germinal vesicle. In the latter case a correction could be applied, if the number of converted oocytes was low.

Control experiments on fertilizability were always performed. They proved that the fertilization percentage was always remarkably low, rarely over 50 per cent and often around 20 per cent. This may partly be due to a lowered mobility on the part of the sperms (reason unknown) and partly to improper sea water conditions (see 2.2). Earlier investigators have stated that 100 per cent fertilization can only occur in connection with natural spawning, artificial fertilization always giving a poor yield.

It may be noted that depriving the *Ast.* cells of their jelly hull has as negligible an effect on the oxygen consumption figure as has the same procedure on *Ps.* cells. Thus an experiment with three parallel divers on naked and on coated *Ast.* eggs from the same female gave the average consumption figures of 2.31 and 2.39×10^{-4} µl. per egg and hour, respectively.

3.122. *Respiration of oocytes and eggs*

Even in *Ast.* cells there is a decreasing oxygen consumption rate as has already been described for ripe *Ps.* eggs (cf. 3.112.1). The rate of decrease is not so steep, however, as in *Ps.* Thus to obtain comparable figures, it ought to be possible to use measurements nearer the point of removal from the ovary than in *Ps.* Nevertheless, the transformation of oocytes into eggs described in the preceding chapter makes early measurements impossible. In the second hour after removal this transformation is practically completed. It is thus most convenient to start evaluating measurements about 120 minutes after removal. The time span of the evaluated measurements has, in the case of the *Ast.* experiments also been about 2 hours. This puts the average point of time used for comparison between different experiments (cf. 3.112.2) to 180 minutes after cell removal from the ovary. The same point of time has been used for both oocytes and eggs.

In order to investigate the cell respiration inside the ovary, experiments on

cells in ovarial fluid were carried out in complete accordance with those on *Ps.* material (cf. 3.113). The difficulties met with in *Ps.* were not at all present in *Ast.* Firstly, the ovarial cells are, without exception, oocytes which make any secondary selection unnecessary. Secondly, the *Ast.* cells are not so densely packed in the ovary, and amoebocytes, etc., are far fewer. Moreover, the form and comparative strength of the ovary makes it easier to free it from sea water or coelomic fluid on the outside. These experiments were started, however, so late in the spawning season that but few can be recorded here. They merely show a general oxygen consumption similar to that of oocytes in sea water.

The results on oocytes and eggs in sea water and on oocytes in ovarial fluid are given in Table V.

Perhaps it is justifiable to think that the results reported in this chapter indicate that the oocytes inside the ovary have a relatively high oxygen consumption velocity owing to the external supply of metabolically utilizable substrate from the ovary.

TABLE V

Average rate ($\bar{r}$) of oxygen consumption of oocytes and eggs of Asterias 180 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Cell type	$\bar{r}$ (µl./cell/hour)	σ	ϵ	n
Eggs in sea water	2.50×10^{-4}	0.33	0.08	18
Oocytes in sea water	1.15×10^{-4}	0.28	0.09	10
Oocytes in ovarial fluid	1.36×10^{-4}	—	—	2

When shed in sea water (or taken out of the ovary) and thus cut off from this supply the rate gradually drops. On transformation into secondary oocytes the rate is considerably raised, but even in this state there is a gradually diminishing oxygen consumption rate.

The difference between eggs and oocytes in sea water is unquestionable (difference = 1.35; $\epsilon_{diff.} = 0.12$). Tang (1931) using Warburg technique (23° C.) arrived at a considerably lower respiration figure for *Asterias* eggs, viz. 1.14×10^{-4} µl./egg and hour (converted to be comparable to the figure in the present investigation), than that obtained in this investigation. Possibly Tang used another species (species not stated; *forbesii*(?), Woods Hole). Tang's value of the size of the egg ($d = 160\,\mu$) does not, however, differ appreciably from that of *Ast.* (cf. 3.123). It might therefore be natural to conclude that the two consumption values ought to have agreed. The rapid shaking rate of the Warburg vessels in Tang's experiments might probably have caused damage to the eggs. Moreover, Tang could not find any difference in the rate of respiration between eggs and oocytes. Boell and co-workers (1940) using diver technique were also unable to state any difference between eggs and oocytes. The close similarity between their experimental conditions and those of the present investigation makes their results quite inexplicable. (Boell and co-workers have hitherto only published their results as a preliminary note.) On the other hand Brooks (1943) in Warburg

experiments on *Asterias forbesii* finds a lower oxygen consumption in oocytes than in eggs.

3.123. *Size of cells*

The size of the *Ast.* egg is given in Table VI. Just as in *Ps.* (cf. 3.114) and in *Par.* (Lindahl and Holter, 1941) the oocytes are slightly smaller than the eggs, as can be seen from Table VII. This difference in volume is, however, definitely too small to account for the found difference in oxygen consumption.

The eggs from the same ovary are often more constant in size in *Ast.* than in *Ps.* The average relative difference (calculated on eggs from 10 females) from the mean diameter were 2.9 per cent ($n = 200$) for *Ast.* and 3.6 per cent ($n = 200$) for *Ps.* eggs.

TABLE VI

Diameter (d) and volume (v) of Asterias eggs
n = number of females examined.
At least 20 eggs from every female were measured.

d (μ)	σ	ϵ	n	v (μl.)
169.1	5.9	1.4	18	25.2×10^{-4}

TABLE VII

Comparison between cell diameters of Asterias oocytes and eggs
Each figure represents the mean of 20 measurements

Female No.	Oocytes	Eggs	$\frac{\text{Eggs}}{\text{Oocytes}}$
1	149.9μ	157.4μ	1.05
2	151.9	157.8	1.04
3	176.0	180.4	1.03
4	156.6	163.1	1.04
5	165.6	169.9	1.03

3.2. *Respiration before and after fertilization*

3.21. *Psammechinus*

In order to follow the oxygen consumption changes induced by activation an attempt was made to perform the fertilization inside the diver during actual measurements. A diver charge type with a diver neck wall drop containing the spermatozoa in sea water was employed (for details cf. "Diver charge Type II" in Borei, 1948). The spermatozoa, however, always crept over the dividing glass surface and gradually reached the eggs before the neck wall drop and the neck seal II were purposely brought together. This premature fertilization was apparently facilitated by the condensed moisture that always prevails on the inside glass surface of the diver. To measure the respiration of the young fertilized egg one is thus obliged to use cells that are fertilized outside the diver. In this case one must be satisfied if the first point of measurement can be obtained at about 40 minutes after fertilization, because charging the diver etc. takes some time.

After the instant rise in oxygen consumption after fertilization, an exponentially increasing part of the respiration curve (Gray, 1926) starts. For the first hours this increase is still quite moderate, thus leaving the curve rather flat. During this time the curve is especially suitable for obtaining comparative values from different egg material. In the actual experiments of this investigation the first evaluated measurement was chosen to be about 60 minutes after fertilization and the last at 180 minutes, thus fixing the point of time for comparison between the different experiments to about 2 hours after fertilization. The results are given in Table VIII.

TABLE VIII

Average rate ($\bar{r}$) of oxygen consumption of fertilized eggs of Psammechinus 120 minutes after fertilization

n = number of diver experiments, each of which was performed on cell material from different females.

$\bar{r}$ (µl./embryo/hour)	σ	ϵ	n
1.84×10^{-4}	0.31	0.06	30

No difference between the two *Ps.* phenotypes could be found (*Ps. Z-form* 1.81×10^{-4} µl., $n = 9$; *Ps. S-form* 1.85×10^{-4} µl., $n = 21$).

Comparing the respiration value of the fertilized egg (Table VIII) with that of the ripe unfertilized egg (Table II) one finds a ratio of 3.6. With *Ps.* eggs Shearer (1922b) using a Barcroft differential manometer technique (14.5° C.) previously found a ratio of 5.7. His measurements on fertilized eggs fall within 30–60 minutes after fertilization. In the case of the unfertilized eggs one cannot judge how long a time elapsed between the removal from the ovary until the measurements were made. This point is certainly of great importance. The earlier the measurements are made the higher the consumption rate of the unfertilized egg will be (cf. Fig. 2). And consequently the higher the rate of the unfertilized egg the smaller the ratio between the oxygen consumptions of the fertilized and unfertilized egg will probably be. In this connection it might be mentioned that the influence of the respiration of the unfertilized egg (at the moment of fertilization) on the future respiration of the fertilized egg is unknown. It is quite possible that a high respiration of the unfertilized egg at the point of fertilization, *i.e.* a still high content of substrate utilizable in the unfertilized state, may be reflected in a relatively higher respiration of the fertilized egg, at least during the first hours.

The value of Borei (1934) on fertilized eggs cannot be used for comparison in this connection because it was referred to the volume of the egg including the fertilization membrane. Owing to the very variable size of this membrane the actual number of eggs in these experiments cannot be calculated.

On material from other sea-urchin species a variety of ratios have been obtained. Only a few will be quoted here. They are mainly taken from the investigations and reviews of Whitaker (1933), Ballentine (1940) and Lindahl and Holter (1941):

Arbacia punctulata	2.6, 3.9, 4.4, 4.5 5.0, 5.0, 5.0, 5.3
Arbacia pustolosa	6.0
Psammechinus microtuberculatus	3.8
Paracentrotus lividus	3.1, 6.0

The cited material is very heterogeneous, representing very different techniques of measurement (such as Winkler titration, Warburg measurements and Cartesian diver experiments) and very differing temperature conditions (15–26° C.). Moreover the actual time after removal from the ovary is never stated for the unfertilized eggs (the most reasonable assumption being that a few hours have elapsed) and in the case of the fertilized eggs the time after fertilization is sometimes uncertain or completely omitted. Thus the significance of the figures is plainly qualitative. They only show that the oxygen consumption of the unfertilized eggs some few hours after their removal from the ovary is considerably lower than that of the fertilized eggs during the first hours after fertilization.

Gray (1926) found that in *Ps.* the rise of oxygen consumption after fertilization followed an exponential curve. Lindahl and Öhman (1938) working on *Par.* were able to confirm this, and Lindahl (1939) established the S-shaped curve characteristic for the oxygen consumption until hatching. After this there is a sudden steep rise associated with the onset of further growth and morphological modifications.

This shape of the respiration curve after fertilization has been perfectly confirmed in this investigation by diver experiments, which are presented in Figure 3. For comparison the curves of Gray, Lindahl and Borei have been plotted on a relative scale (putting the values 30 minutes after fertilization alike) in the small right hand bottom graph. To complete the survey the curve of the correspondingly run diver with unfertilized eggs has also been plotted in the main graph. In this the times of the first mitoses (judged from microscopically observed parallel cultures) and those of ciliation, hatching etc. have also been indicated. It must be observed that the time which elapses before the hatching of the embryos is somewhat longer inside the diver than in parallel ordinary cultures, viz. 9 and 8.5 hours, respectively (cf. 3.3). This delay is always observed and must be ascribed to the relatively dense packing inside the diver. If the embryos, after hatching, are washed out of the diver and cultured in the ordinary way they develop into perfectly normal plutei.

Zeuthen (1947b) working with refined diver technique on *Ps.* (16° C.) was able to show that the primary exponential oxygen consumption curve has waves superimposed upon it. The minima of these waves correspond to the first cleavages (telophase) of the egg. His main graph must, however, be incorrect as to the time position of the cleavages, *e.g.* in the main graph the fifth mitoses is indicated at 5 hours after fertilization, whereas it correctly occurs at about 3 hours and 20 minutes. Assuming, however, that in his small graph the time mark between the fourth and fifth mitoses stands for 3 hours after fertilization the values are in concordance with known cleavage rates. (In Figure 3 of the present paper the first cleavages are marked in their right position on the curve). Moreover, in order to get an entire graphical scheme of the oxygen consumption of the reproduction cell of the female sea-urchin, Zeuthen has completed his *Ps.* curve with the results of Lindahl and Holter (1941) on oocytes and unfertilized eggs of *Par.*, and with

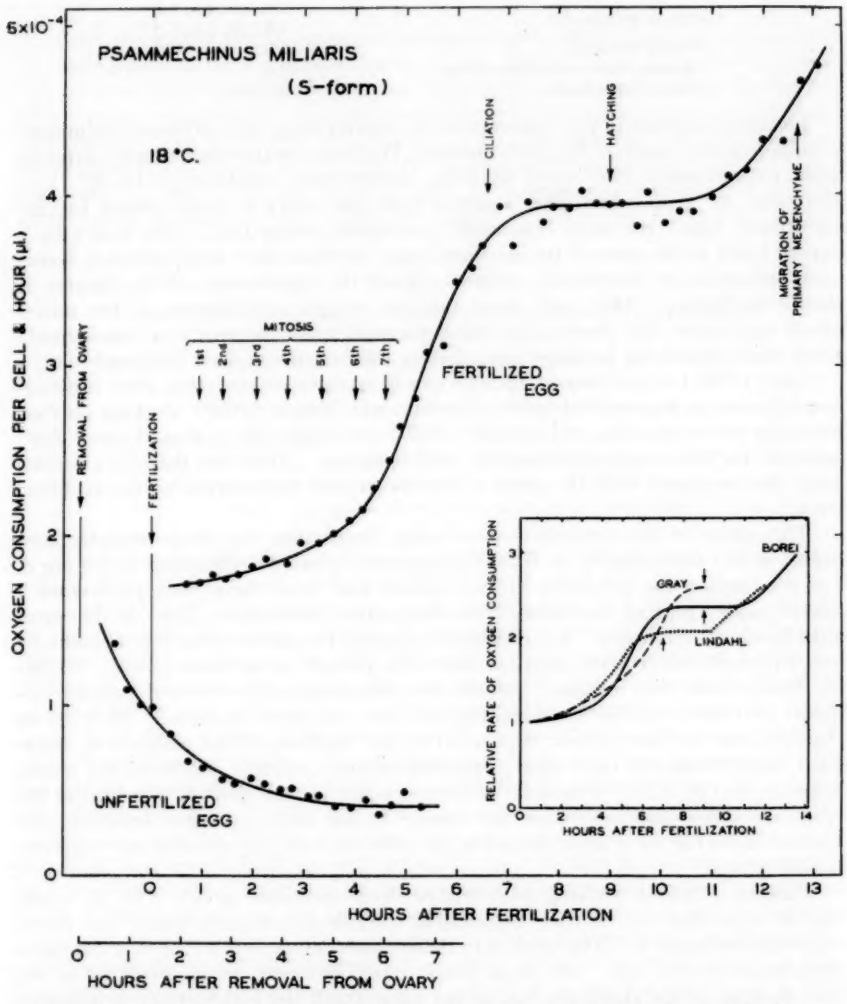

FIGURE 3. Oxygen consumption of *Psammechinus* eggs before and after fertilization. The values are obtained from parallel divers, one with unfertilized, the other with fertilized eggs. For comparison the results of Gray (1926) on *Psammechinus miliaris* (17° C.) and of Lindahl (1939) on *Paracentrotus lividus* (22° C.) together with those of this investigation (18° C.) are plotted on a relative scale (extrapolated values at 30 minutes after fertilization = 100) in the small right hand bottom graph. In this graph the times of hatching are indicated by arrows. In the main graph the position of the telophase (appearance of cleavage furrow) of the earlier mitoses is indicated.

the results of Lindahl (1939) on fertilized eggs of *Par.* From the results on *Ps.* oocytes and unfertilized *Ps.* eggs presented in this paper (cf. 3.112 and 3.113) it is apparent that the respiration of the *Ps.* and *Par.* cells differs. Thus results from the two species must not be represented as a continuation in one and the same curve.

As has been previously mentioned it has not been possible to use the diver technique to measure the respiration until 40 minutes after the fertilization. Before this time there is said, however, to be a higher oxygen consumption than at any time during the next few hours. This was first indicated in measurements of Shearer (1922a) on *Psammechinus microtuberculatus* and later studied in detail by Runnström (1933) and by Laser and Rothschild (1939) working on *Par.* and *Ps.*, respectively. Nevertheless the exact shape of this part of the curve is still uncertain. (The slight temporary rise over the unfertilized egg value immediately after fertilization, which is indicated in Zeuthen (1947b) in his main graph, is not drawn in accordance with the findings of the mentioned investigators.) The period between fertilization and the first diver measurements has been left empty in Figure 3, but one must, however, keep in mind that during this period oxygen consumption rates, higher than that at 40 minutes, may have occurred.

3.22. *Asterias*

Comparing the respiration rates of unfertilized and fertilized *Asterias* eggs Loeb and Wasteneys (1912) (Winkler measurements) and Tang (1931) (Warburg technique) found no differences. In Cartesian diver experiments Boell and co-workers (1940) confirmed this. The results in the present paper in no way differ from these results. Here the respiration has been followed, however, for a longer space of time than in the earlier investigations, namely over a period of more than 200 minutes after fertilization, *i.e.* over the first mitosis. A gradual increase in oxygen consumption rate is to be noted during this time, as will be seen from Figure 4 (concerning the cell material, cf. 3.121).

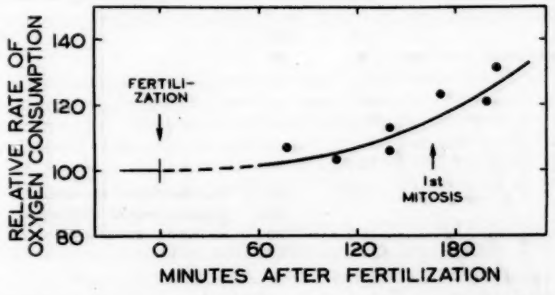

FIGURE 4. Oxygen consumption of *Asterias* eggs before and after fertilization.
The exact position of the first mitosis telophase in relation to the time of fertilization depends on the state of the egg when fertilized (secondary oocyte or egg). In a batch of cells mitosis may thus not occur synchronously.

3.3. *Cleavage rate*

In previous chapters reference has been made to the succession of mitoses and the time of their occurrence after fertilization. Though this cleavage rate in *Ps.* has repeatedly been studied by various authors, it has, for the sake of control, been checked even in this investiga-

TABLE IX

Cleavage rate of fertilized Psammechinus eggs

Concerning right time values in Zeuthen's experiments cf. 3.21

Author	Gray (1926)	Zeuthen (1947b)	Borei (this investig.)
Temperature	17° C.	16° C.	18° C.
Salinity	34–35‰	32‰	33.2‰
	Time after fertilization:		
1st mitosis	67 mins.	—	56 mins.
2nd	100	—	83
3rd	132	—	127
4th	168	165 mins.	160
5th	203	200	~200
6th	238	240	~240
7th	271	(290)	—
Hatching	9 hours	9 hours 30 mins.	8 hours 35 mins.

tion. Found discrepancies were, however, with exception of the first cleavage time, of minor importance as is shown in Table IX.

It should be noted that in this table the values of Borei and Gray were obtained from cultures, whereas Zeuthen's are from observations on dividing eggs inside a diver. In this latter case the hatching time is somewhat delayed. As has been mentioned before (3.21) even in the present investigation a minor delay has been observed in cells when inside a diver.

In Figure 5 the cleavage sequence for *Ps.* and *Ast.* has been represented graphically. For comparison the cleavage rate for the irregular sea-urchin *Echinocardium cordatum* has been plotted in the same figure. All values were obtained during this investigation.

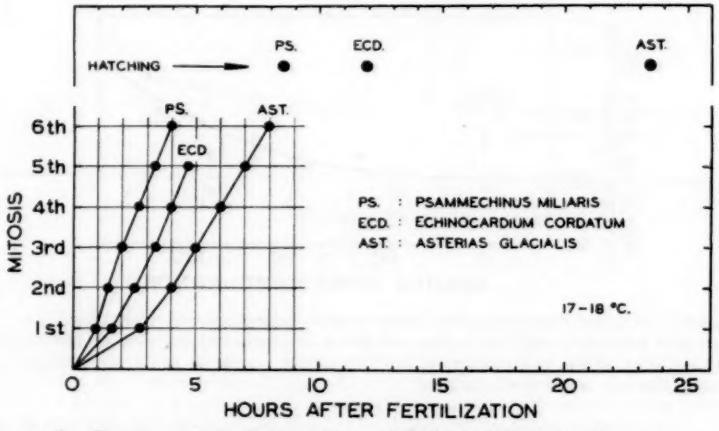

FIGURE 5. Cleavage rate in *Psammechinus, Asterias* and *Echinocardium.*

Values obtained from observations on sparse cultures (200 eggs in 10 ml. sea water). The mitoses are represented from the appearance of the cleavage furrow (teleophase). The position of the first cleavage in *Ast.* is dependent on the state of the egg when fertilized; in the represented sequence the germinal vesicle had just broken down when the sperms were added.

4. General Discussion

Many investigators consider that they have reason to look upon the mature sea-urchin egg as a resting cell between the growing oocyte and the developing fertilized egg. (For literature see Lindahl and Holter, 1941.) The oxygen consumption measurements with diver technique performed by Lindahl and Holter (1941) on *Par.* oocytes, mature unfertilized eggs and fertilized eggs seem to give excellent support to this view. They found that the primary oocytes apparently have a respiration even slightly higher than that of the newly fertilized egg, whereas the unfertilized eggs have a considerably lower oxygen consumption. The authors compare the state of the unfertilized egg with the diapause of certain insect eggs. The investigations of Whitaker (1933) indicate that the low unfertilized egg respiration in comparison with that of the fertilized egg is, among marine invertebrates, peculiar to the sea-urchin group.

The experiments in this paper show, however, that the sea-urchin group is not quite homogeneous in respect to oocyte respiration, as may be seen from Figure 6. Though the oocyte oxygen consumption was measured in principally the same way as in Lindahl and Holter's investigation it was found that *Ps.* oocytes, in contradistinction to those of *Par.*, consumed oxygen at a rather low rate, not differing very much from that of the unfertilized egg.

A new fact, however, is revealed in this investigation concerning the sea-urchin egg respiration, viz. the rapidly decreasing rate of oxygen consumption after the cell has been removed from the ovary (cf. Fig. 2). This declining respiration rapidly and asymptotically approaches a considerably lower value than the initial one (cf. parallels in the endogenous respiration of baker's yeast, kinetically studied by Borei, 1942, and briefly reviewed in 3.112.1). According to these facts the initial rate of oxygen consumption upon egg removal may even be higher than the rate of the fertilized egg during the first hours after activation (cf. Fig. 3). Such a rapidly declining respiration has also been found to be characteristic of the *Ps.* oocytes (3.113).

In all previous investigations the cell material must undoubtedly, though never stated, have been at least some hours old (reckoned from the time of removal from the ovary) when used in consumption measurements. Thus in the present investigation only such *Ps.* and *Ast.* measurements as were obtained from material of that age may be compared with the values of the previous authors. This means that only the measurements on the low level stage which are given in 3.112.2, 3.113 and 3.122 and summarized in Figure 6 may be used for comparison.

If differences now exist, as they probably do, both between the species and between the different kinds of cells within the species, as regards the rapidity with which the low level state is reached, *i.e.*, in steepness of the declining curve, it might in the *Par.* case be that one has hit upon oocytes which show a very slow decrease, whereas in the *Ps.* case the oocytes show a rapid decrease. Under such circumstances the apparent contradictions between *Ps.* and *Par.* oocyte respiration can be understood.

In the case of Boell and co-workers' (1940) findings in *Arbacia* of a lower respiration in the oocytes than in the eggs, it might be either that the oocyte respiration decrease is especially rapid or that the respiration decrease of the eggs is especially slow in that species, or it might be that the time which has elapsed since the removal from the ovary is longer for the oocytes. As no technical points

whatsoever are stated in Boell and co-workers' paper it is impossible to draw any conclusions.

It is very interesting to discover that there is general agreement within the sea-urchin group as to a low respiration in the unfertilized egg (when measured some hours after removal from the ovary) in comparison with the respiration of the fertilized cell. (The reviews of Whitaker (1933) and Ballantine (1940) and the results given in 3.21 might be consulted.) Now Loeb and Wasteneys (1912) have found that unfertilized *Asterias* eggs which respire at the same rate as the

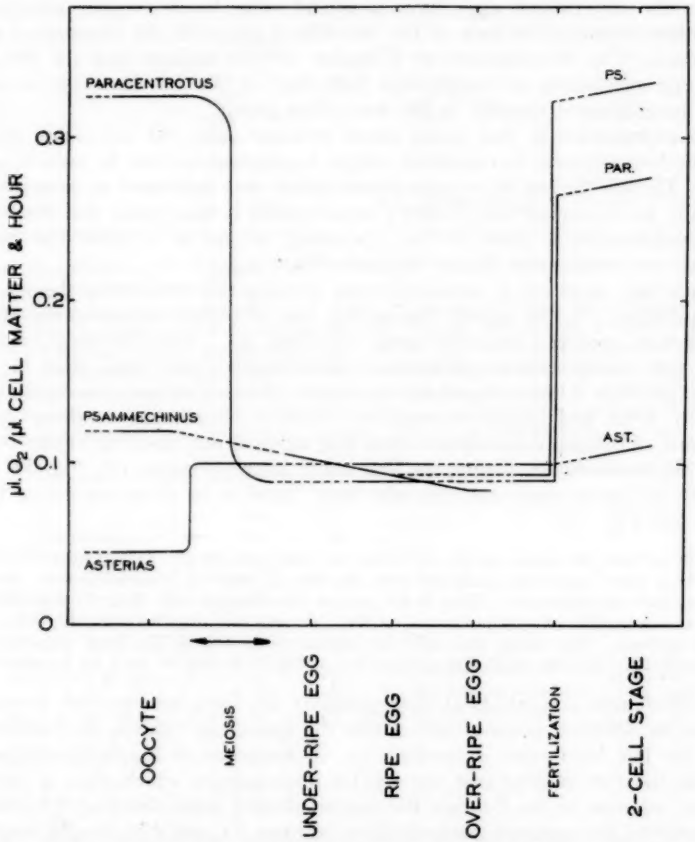

FIGURE 6. Comparison on a cell volume basis between the oxygen consumption of different female reproduction cells from *Psammechinus*, *Paracentrotus* and *Asterias*.

The results on *Ps.* and *Ast.* are from the present investigation; those on *Par.* from Lindahl and Holter's (1941). In the unfertilized cells the oxygen consumption was always measured several hours after cell removal from the ovary. All rates are uniformly calculated for 18° C. and expressed as if measured at 0° C. and at normal barometric pressure. Average cell volume values used: *Ps.* 5.56×10^{-4} µl., *Par.* 5.75×10^{-4} µl. and *Ast.* 25.2×10^{-4} µl.

fertilized ones, die very rapidly if not kept anaerobically (*i.e.*, if the oxidative processes within the eggs are allowed to continue at an unlimited high level). In view of this and the considerations of other authors one is inclined to look upon the low respiration of the sea-urchin egg as a natural precaution in order to facilitate long life for the shed egg. In this connection one must consider the actual conditions of the localities at time of spawning. The animals there live rather close together and spawning probably sweeps epidemically and simultaneously over the specimens, both male and female, of a given species in a community. Thus a rapid fertilization may be secured, which would mean that the egg respiration would only have time to enter the very first part of the declining curve. It is possible that eggs, which accidentally are not fertilized at once, are preserved for future activation through this low-level mechanism, which thus promotes the reproduction possibilities of the group. On the other hand, however, the similar declining curve of the oocyte respiration will probably have no biological significance whatsoever because, as is generally agreed, the sea-urchin oocytes are never shed.

The biochemical mechanism that causes the differences in respiration between fertilized and unfertilized sea-urchin eggs, as studied by Runnström (1930, 1933, 1935), Örström (1932), Korr (1937) and others, is likely to be characterized in the unfertilized egg by a block in the chain of carriers which, in the fertilized egg, mediates the oxidation of the substrates. This chain of carriers is supposed to include the cytochrome-system. Runnström considered that the oxidase was unsaturated with its substrate, and later investigations have furthered the view that substrates, dehydrogenases and oxidase are kept apart in the unfertilized egg. It is remarkable that the respiration of the unfertilized egg is insensitive to inhibitors affecting the cytochrome-system, whereas the respiration of the fertilized egg is very sensitive. In all these investigations it has been pointed out that the respiration of the unfertilized egg is low in comparison with that of the fertilized egg. This means that the studies have been performed on unfertilized eggs, in which the declining respiration part has already reached a low level. As the initial respiration of the unfertilized egg (when just removed from the ovary) can be even higher than that of the fertilized egg, the question therefore arises as to the relation between the rapidly declining (monomolecular) respiration part of the unfertilized egg and the respiration of the fertilized egg. Whether or not the oxidase is as important for this part of the respiration of the unfertilized egg as it is for the fertilized egg can probably be settled if inhibition experiments are performed as early as possible after the eggs have been removed from the ovary, *i.e.* when the declining respiration is still prominent.

The oxygen consumption figures in the three stages of ripeness of the unfertilized *Ps.* egg reveal that the respiration value slightly decreases with growing ripeness (cf. 3.113 and Fig. 6). It has not been possible to establish any difference in the early declining respiration of these stages. The material is, however, too small for convincing interpretations. (It should be kept in mind that the respiration of unfertilized sea-urchin eggs when kept for a long time in sea water undergoes a change, thus becoming more like that of the fertilized egg.)

In the case of the *Ast.* egg it has been found that the respiration is lower in the primary oocyte than in either the secondary oocyte or the unfertilized egg (cf. 3.122 and Fig. 6), but this finding is in contradiction to earlier ones (Tang, 1931, and Boell and co-workers, 1940; cf., however, Brooks, 1943). As the eggs on natural

spawning are shed with broken down germinal vesicle, *i.e.* as secondary oocytes or as eggs (cf. Runnström, 1944), the jump in respiration rate between primary and secondary oocytes does not occur outside the ovary. The respiration after fertilization proceeds (as has been reported by several previous investigators) at much the same level as before. As the decreasing part of the respiration of the cells after their removal from the ovary is not very marked in this material, the rate of respiration of the fertilized eggs may not, under natural conditions, differ very much from that found in this investigation (3.22), even if the fertilization takes place very soon after spawning. Thus if the rates of respiration of the fertilized sea-urchin eggs and the fertilized starfish eggs are compared on a cell volume basis (see Fig. 6), a very much lower respiration in the case of the starfish will be found. Whitaker (1933) has argued that the oxygen consumption of fertilized marine animal eggs from several invertebrate phyla and even of other developing cells, when compared on a cell volume basis, will show a remarkable consistency, whereas the respiration values of the unfertilized cells are widely scattered. It may be read from Whitaker's discussion, though not stated in these terms, that the respiration of the fertilized eggs is thought to be intimately connected with the work of morphogenesis and with biochemical activities connected with growth, and that in developing cells the amount of oxygen required for these purposes is about equal per volume of cytoplasmic matter. It is stated, however, that big cells, especially yolky eggs respire at a much lower rate. Other factors to be considered will, no doubt, be different degrees of cytoplasmic hydration, inert inclusions in vacuoles, etc., dead protecting or otherwise supporting structures, etc. The measurements by Tang (1931) on *Ast.* egg respiration before and after fertilization gave too low values to fit in Whitaker's scheme. The latter author severely criticizes technical weaknesses in Tang's measurements and leaves them out of his survey. The present investigation has certainly found Tang's values to be notably low (cf. 3.122), but still the fertilized *Ast.* egg respiration is remarkably low in comparison with that of the sea-urchin egg (cf. Fig. 6). It should be borne in mind that the *Ast.* egg has a volume that is about 5 times greater than that of the *Ps.* or *Par.* egg. This fact may probably, in the light of the discussion on the connection between body size and metabolic rate recently put forward by Zeuthen (1947a), be of more importance than considerations concerning supposed morphogenetic work.

To summarize the egg respiratory conditions found in this investigation together with those previously known, the graphical schemes in Figure 7 may serve.

The *Ps.* ripe egg (Fig. 7a) (cf. 3.112.1) has very probably a high respiration level in the ovary; at least it starts with high respiration velocity when brought into sea water. The oxygen consumption rate rapidly decreases and within some hours reaches a low and fairly constant level. The oocyte behaves similarly. At fertilization the rate immediately rises to around the level of the just removed egg, drops slightly and thereafter proceeds after an exponentially increasing curve (cf. 3.21). After natural spawning, fertilization probably occurs very soon, thus leaving the decreasing curve without much importance. Only ripe eggs are fertilizable.

The *Ast.* primary oocyte oxygen consumption rate (Fig. 7b) (cf. 3.122) decreases comparatively slowly in sea water. If the oocyte is ripe it soon starts the first meiosis, thereby increasing its respiration considerably. The second meiosis soon follows and a slow decrease in oxygen consumption continues until fertiliza-

tion occurs. After fertilization the consumption proceeds as an exponentially increasing curve (cf. 3.22). On fertilization no sudden jump in consumption rate occurs as in *Ps.* When at natural spawning the cell leaves the ovary its nuclear membrane has disappeared. Thus the jump in the rate of respiration on transformation from primary to secondary oocyte has already occurred. Even oocytes may be fertilized, but then the time which elapses before the first mitosis will be longer thus allowing meioses to occur and thereafter the resting sperm nucleus to unite with the egg nucleus.

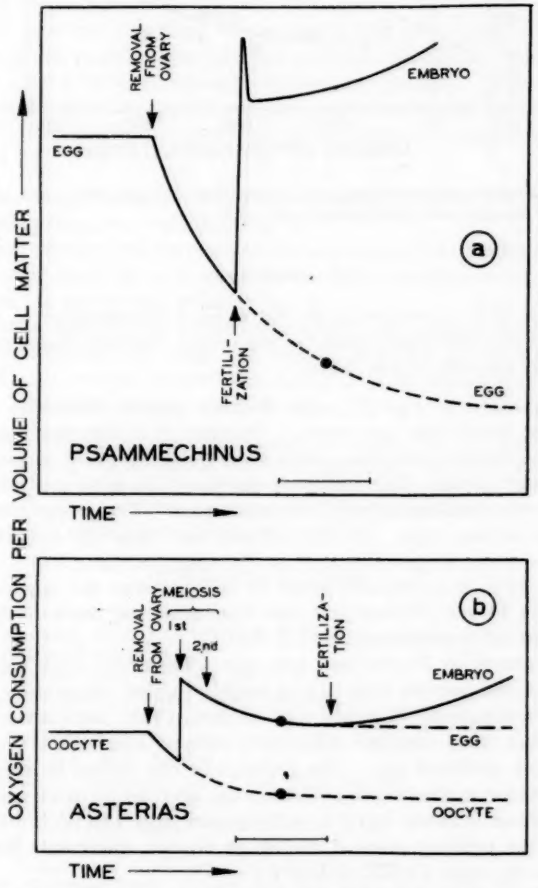

FIGURE 7. Generalized schemes of oxygen consumption per cell volume of reproduction cells of *Psammechinus* and *Asterias.*

Time span of evaluated diver measurements as well as location of comparison value on respiration curve indicated for each cell type.

It is interesting to note that in fertilized eggs of both *Ps.* and *Ast.* the increase in oxygen consumption, which takes place during the first hours of development after fertilization, seems to be of the same exponential type in both species. This will be seen from Figure 8, where values from the first 4 hours' development in both species have been plotted on a relative scale putting the values at 40 minutes after fertilization = 100.

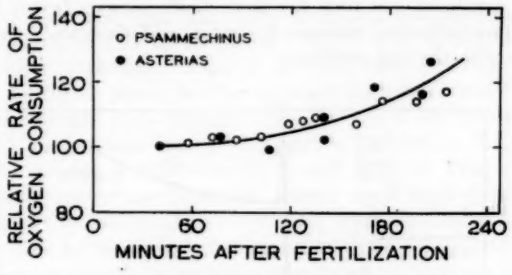

FIGURE 8. Relative oxygen consumption of fertilized *Psammechinus* and *Asterias* eggs. Values at 40 minutes after fertilization put = 100.

5. Summary

In Cartesian diver experiments on the oxygen consumption of oocytes, unfertilized eggs and fertilized eggs from the sea-urchin *Psammechinus miliaris* and the starfish *Asterias glacialis* it was found:

1. The respiration of ripe *Ps.* eggs declines rapidly after they have been removed from the ovary into sea water. Starting at a rate that may exceed that of newly fertilized eggs it has thus, after some hours, attained a comparatively low and fairly constant level. The declining curve on kinetical analysis proves to be composed of a monomolecular and a constant part. The respiration curve of *Ps.* oocytes is of a similar type. In *Ast.* oocytes and eggs the respiratory decrease, though present, is not so prominent as in *Ps.* cells (3.112.1, 3.113, 3.122, Fig. 2).

2. Though there is a real difference in size between the eggs of the two *Ps.* phenotypes (the littoral *Z-form* and the *S-form* of the depths) no difference is found in the rate of respiration (3.112.2, 3.114).

3. Measurements on *Ps.* oocytes and eggs some hours after removal from the ovary show that the oocytes have only a slightly higher respiration than the eggs. The earlier investigations (Lindahl and Holter, 1941) on *Paracentrotus lividus* eggs showed that these oocytes maintain a rate of respiration even higher than that of the newly fertilized egg. The findings in *Par.* might be ascribed to a slow respiration decrease in the oocytes, whereas the decrease is more rapid in the eggs. In *Ps.* the decrease is about equal in oocytes and eggs (3.112.2, 3.113, 4, Fig. 6).

4. In *Ast.* the primary oocytes respire at a much lower rate than do the secondary ones or the eggs (3.122, 4, Fig. 6).

5. In *Ps.* there is a gradual slight decrease in egg respiration with advancing cytoplasmic maturity (3.113).

6. In both *Ps.* and *Ast.* the respiration of oocytes in ovarial fluid seems to be of the same order of magnitude as that of oocytes in sea water (3.113, 3.122).

7. The shape of the respiration curve in *Ps.* after fertilization is in full concordance with earlier results obtained with different techniques by Gray (1926) and Lindahl (1939) (3.21, Fig. 3).

8. The value of the rise in respiration, that occurs in sea-urchin eggs on fertilization, may entirely depend on where on the slope of the decreasing egg respiration curve fertilization occurs. (This rise is characteristic for sea-urchin eggs and has repeatedly been found by earlier investigators.) It is thought that on natural spawning the rise is rather feebly marked because of early fertilization, and that correspondingly the low level respiration of the unfertilized egg may not be reached (3.21, 4, Figs. 3 and 7).

9. In *Ast.* there is no immediate rise in respiration after fertilization, but there is a gradual rise which exactly resembles the exponential increase in newly fertilized sea-urchin eggs (after the first sudden increase has passed). The rise from the oocyte respiration level to that of the egg will, under natural conditions, not occur outside the ovary, as the cells are shed with broken down nuclear membranes (3.22, 4, Figs. 4, 7 and 8).

Cleavage rates are given up to the sixth mitosis for *Ps.*, *Ast.* and *Echinocardium cordatum;* hatching time is noted (3.3, Fig. 5).

It is discussed whether the decrease in respiration of the unfertilized sea-urchin egg after its removal from the ovary has any possible significance for the biochemical aspects of the sea-urchin egg respiration (4).

If the respiration rates found in this investigation are compared on a cell volume basis it is found that the *Ast.* egg will not fit into the generalized scheme of Whitaker (1933) for marine invertebrate eggs; it is discussed why the *Ast.* egg respiration is so comparatively low (4, Fig. 6).

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